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THE JOURNAL
OF RESEARCH
ON THE
LEPIDOPTERA

VOLUME 45 2012

ISSN 0022 4324 (PRINT) 2156 5457 (ONLINE)
THE LEPIDOPTERA RESEARCH FOUNDATION

PUBLISHED BY: The Lepidoptera Research Foundation, Inc.
9620 Heather Road
Beverly Hills, California 90210-1757
TEL (310) 274 1052

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	Regular (Individual)	\$ 25.00 year
	Contributing	\$ 30.00 or more year
	Subscription Rate/ Institutions	\$ 35.00 year
	Life	\$ 300.00

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Characteristics of monarch butterflies (*Danaus plexippus*) that stopover at a site in coastal South Carolina during fall migration

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Abstract. While the annual fall migration of eastern North American monarch butterflies (*Danaus plexippus*) to wintering sites in central Mexico is a well-known and frequently-studied phenomenon, one aspect of this behavior that remains poorly understood is the nature of their migratory stopovers. Like migrating birds, monarchs must stop frequently during their journey to rest and refuel (i.e. obtain food), and why they choose to stop and for how long are important pieces of information, yet these have rarely been examined for monarchs. In this study we utilized data from a long-term monarch migration tagging operation in South Carolina to address certain aspects of this knowledge gap. Monarchs are tagged at this site each fall and recaptured individuals are also noted. Here we compared the characteristics of these recaptured individuals (n=407 over 13 years) to those that were never recaptured (n=12,989), focusing specifically on their wing size and wing condition, which was scored on a 1-5 scale. We also looked for evidence that stopover lengths are influenced by size or condition. The overall recapture rate at this site was 3.1%, although there was a small degree of annual variation in this rate (ranging from 1.3 - 5.6%). Males were recaptured twice as often as females. Recaptured monarchs did not differ from non-recaptured monarchs in wing size, but did have greater wing damage and wear than non-recaptured individuals. The recapture rate was the highest (8.5%) for monarchs with the most worn and damaged wings, while the rate was the lowest (2.9%) for monarchs with the freshest wings with no damage. Furthermore, monarchs with highly damaged and worn wings tended to remain longer at the stopover site than those with no damage or wear. Taken together, these results indicate that wing condition influences whether or not monarchs remain at a stopover site and for how long. In addition, they suggest that monarchs with poor wing condition may have a slower pace of migration owing to their more frequent and longer stopovers.

Key words: Monarch butterfly, *Danaus plexippus*, migration, stopover, tagging, wing condition.

INTRODUCTION

Among the Lepidoptera, monarch butterflies (*Danaus plexippus*) in eastern North America are unique because of their spectacular, annual migration to a series of overwintering sites in the mountains of central Mexico. Each fall, the last summer generation

of monarchs undertakes this journey, which lasts over two months, and spans over 3000km (Oberhauser & Solensky, 2004). Like migratory birds, monarchs must make frequent stops along the way ('stopovers') to rest and/or forage for food (nectar), which is transformed into fat reserves (Brower *et al.*, 2006). As it is with migrating birds (e.g. Moore *et al.*, 1995; Hutto, 1998; Mehlman *et al.*, 2005), such stopovers are likely extremely important to the successful migration of this cohort, since monarchs utilize accumulated fat reserves both as fuel for the migratory flight, as well as to sustain themselves during the overwintering period (Alonso-Mejia *et al.*, 1997). Despite their obvious importance however, there has been very little empirical research on the nature of monarch stopovers (Davis & Garland, 2004). As such, we still have only a rudimentary understanding of how stopover sites are utilized by monarchs.

As in ornithological research projects, to study stopover of migrating monarchs, individuals must

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Received: 19 December 2011

Accepted: 17 January 2012

be captured, marked, and an effort must be made to re-observe or recapture the marked individuals at the stopover site (Davis & Garland, 2004). This allows researchers to identify the individuals that stay at the site for a lengthy period (a long stopover), versus those that are not recaptured, and are assumed to have left shortly after initial capture. Recapturing individuals also allows for estimation of the duration of stopover (i.e. the number of days between initial capture and recapture), which in itself can provide information as to the importance of the resources at the site (e.g. Cherry, 1982; Moore & Simons, 1992; Morris *et al.*, 1996; Carlisle *et al.*, 2005), and the individual variation in energetic requirements. For example, when a migrating bird's fat stores become depleted, it must stop and attempt to replenish them by foraging. And, the length of time it spends at a given site is directly related to the amount of fat it must acquire before resuming flight (e.g. Cherry, 1982; Winker *et al.*, 1992; Davis, 2001; Jones *et al.*, 2002). Thus, the stopover decisions of birds (whether to stop and for how long) are highly influenced by the condition of the individual.

Unlike migrating birds, the overall condition of a butterfly is partly dependent on the integrity of its wings. Like most butterflies, monarchs can sustain damage to their wings over time, for a number of reasons, including bird strikes, mating struggles, close contact with conspecifics during roosting, or general wear and tear with age (Leong *et al.*, 1993). Regardless of the reason, wing damage can affect flight ability in some insects (Combes *et al.*, 2010; Dukas & Dukas, 2011), and for monarchs, this could ultimately influence overall migration success. Consider that if a monarch with damaged wings has difficulty foraging, it may have to spend more time at any given stopover site to meet its energetic requirements (i.e. fat stores) for the next flight. And, if this happens at multiple stopover sites, damaged individuals would eventually fall behind. Moreover, individuals with damaged wings may also expend more energy flying, and may therefore require more frequent stopovers than would undamaged individuals. For multiple reasons then, wing 'condition' could be an important influence on stopover decisions of monarchs.

A recent study uncovered a surprising and inexplicable facet of monarch migration, that in the last 30 years, the proportion of females in the migratory generation appears to be declining (Davis & Rendon-Salinas, 2010), such that in recent collections of migrating monarchs, females make up approximately 30-35% of the migrating cohort (Brower *et al.*, 2006; Brindza *et al.*, 2008; McCord & Davis, 2010). The cause of this pattern remains

unclear, but since sex ratios on the breeding grounds tend to be close to 50-50 (Herman, 1988), it is possible that something is occurring in recent years to selectively remove females from the population during the southward migration. One possibility is that females may stopover more frequently, and/or spend more time at stopover sites than do males, which, as in the case with damaged individuals, would lead to a longer overall migration, and hence greater opportunities for mortality.

The current study aims to enhance understanding of stopover biology of monarch butterflies by examining data from a long-term tagging project at a stopover site in South Carolina (McCord & Davis, 2010). Each fall since 1996, migrating monarch butterflies have been captured at this site by the lead author, who tags all butterflies (with numbered MonarchWatch stickers) and takes detailed notes on all captured individuals. By the end of 2008, he had tagged over 12,000 individuals, and importantly, he also has records of all recaptures at this site. Using these data, general patterns of migration have been examined already (McCord & Davis, 2010). Here, we use these data (from 1997-2008) to examine in detail the individuals that were recaptured by JWM after initial capture and release. These individuals represent monarchs undertaking a stopover. Thus, the primary goal of this project was to determine the characteristics of monarchs that stopover at this site. Specifically, we asked do these monarchs differ in terms of size (wing length) or wing condition from those monarchs that did not remain at the site (i.e. that were not recaptured), and of those that were recaptured, was their stopover duration influenced by either their size or their wing condition? We also looked for possible gender differences in stopovers (i.e. likelihood of stopover and/or duration of stopover as linked to gender of monarchs), that might explain the apparent male-biased sex ratio of the migratory cohort.

METHODS

Study site

The data from this project come from a long-term study of monarch butterfly migration at Folly Beach, SC (32.6°N, -79.9°W), which is an approximately 10 km×1 km barrier island, oriented northeast—southwest, on the central South Carolina coast in Charleston County. Folly Beach is connected to the mainland (James Island) via the SC highway 171 causeway and is approximately 15 km from the city of Charleston. The vast majority of the island is under residential

development with a small, localized business district near the island center. Further details of the study site, including habitat features, are given elsewhere (McCord & Davis, 2010).

Capturing monarchs

All monarchs were captured by the primary author (JWM), who lives on James Island and approximately 10 km from Folly Beach. Each year from August through December he captured (with a butterfly net) as many migrating monarchs as possible for the purposes of tagging them with uniquely numbered stickers from the MonarchWatch program (McCord & Davis, 2010). While there was no effort made to standardize the number of hours spent per day collecting (obtaining rates of capture per hour was not a goal of this project), JWM did spend more time collecting when monarchs were abundant at the site, which was typically from mid-October to mid-November (McCord & Davis, 2010). When a monarch was captured, JWM tagged it with a MonarchWatch sticker on the underside of the hindwing, recorded the gender, measured its forewing length and subjectively scored the condition of the wings on a scale of 1-5. In this system, 5 = excellent condition, no, or practically no, wear or damage, 4 = minimal damage/wear that presumably causes little immediate reduction in flying efficiency, 3 = moderate, damage significant enough to likely cause some reduction in flying efficiency, 2 = significant damage (often one wing-tip missing), causing labored flight, and 1 = major damage, flight extremely labored (usually with portion of both forewing tips missing and often with hindwing damage as well; Fig. 1). Importantly, when a previously-tagged monarch was spotted, JWM made every effort to capture it and note the tag number. For the purposes of the current paper, this allowed us to differentiate monarchs that were later recaptured at the site (following initial capture, i.e. 'stopover monarchs') from those that were never recaptured ('non-stopover monarchs'). All monarchs were released at the site of capture.

Data analysis

We initially used data from all captured monarchs to examine several possible factors that could influence whether or not the monarchs were later recaptured. We used logistic regression with the response variable being monarch later recaptured or not recaptured and with the year and gender as categorical predictors, along with wing length and wing condition being continuous covariates.

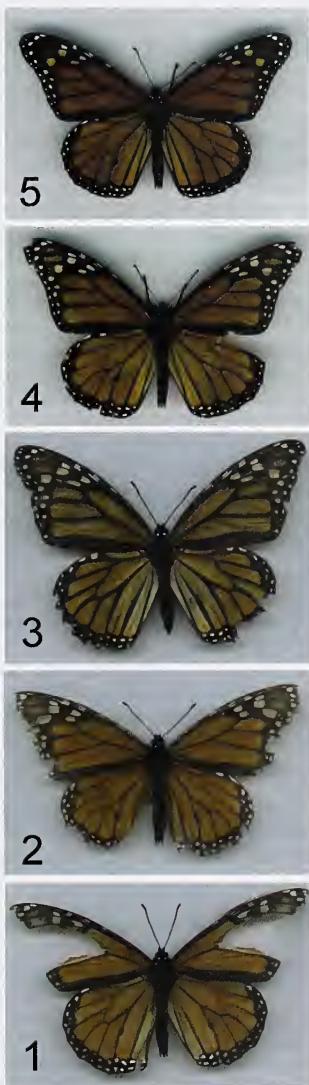


Figure 1. Scanned images of monarch butterflies representing all 5 categories of wing condition used in this project. See methods for description of criteria for assigning scores. Monarchs in category 5 had the freshest wings with no damage while those in category 1 had very worn wings with considerable damage.

Then, we compared the characteristics of monarchs (their size and condition) that were later recaptured to those that were not recaptured using general linear modeling, where wing length or condition (considered as a continuous variable) were response variables and recovery (yes or no) was a predictor, along with gender and year. Next, using the data from all monarchs that were recaptured we calculated the length of their stopover (in days) as the date of recapture minus the date of initial capture, plus 1 day (Davis & Garland, 2004). Because the actual dates of arrival to and departure from our site was not known for each monarch, these stopover 'length' values can be considered conservative estimates; actual stopover lengths are likely greater than what we calculated. To approximate a normal distribution these data were log-transformed. Then, log-transformed stopover length was the response variable in a general linear model that examined if year, gender, wing length or wing condition affected stopover lengths. All data were analyzed using Statistica 6.1 software (Statistica, 2003) and significance was accepted if $p<0.05$.

RESULTS

From 1996 through to 2008, JWM captured and tagged 12,989 monarchs (Table 1). Of these, a total of 407 monarchs (3.1%) were later recaptured by him. The percentage of monarchs recaptured

varied from year to year, with a low of 1.3% in 2000 to 5.6% in 2002. Moreover, results from the logistic regression analysis showed a significant effect of year on the likelihood of recapture ($df=12, \chi^2=55.2, p<0.0001$). There was also a significant effect of gender in this analysis ($df=1, \chi^2=59.3, p<0.0001$); the recapture rate for males (4.3%) was over twice that of females (1.8%), although the magnitude of this difference depended on the year (significant year x gender interaction effect; $df=11, \chi^2=20.8, p=0.036$). Butterfly size (forewing length) was not significant ($df=1, \chi^2=0.8, p=0.348$), however, wing condition was significant ($df=1, \chi^2=21.3, p<0.0001$). The direction of the effect of wing condition on recapture rate can be seen in Table 2, where recapture rates of monarchs in all five wing condition categories are presented. The recapture rate was the highest (8.5%) for monarchs with the most worn and damaged wings (category 1), while the rate was the lowest (2.9%) for monarchs with the freshest wings with no damage (category 5).

In the direct comparison of wing lengths and condition of monarchs that were never recaptured to those that were recaptured, there was no significant difference in wing lengths ($F_{1,12729}=0.05, p=0.823$; Fig. 2A). While the gender and year parameters were not of particular interest in this test, there was an expected effect of gender ($F_{1,12729}=160.63, p<0.0001$; Fig. 2A), and a surprising effect of year ($F_{12,12729}=15.33, p<0.0001$) on wing lengths. The annual differences

Table 1. Summary of monarchs captured during all years, with proportions that were later recaptured (in the same season). The average length of stopover is shown for all years with standard deviations in parentheses.

Year	Total Captured	# Recaptured	% Recaptured	Stopover Length (d)
1996	961	36	3.7	4.8 (5.2)
1997	1,131	44	3.9	4.4 (3.1)
1998	285	8	2.8	7.3 (7.6)
1999	801	21	2.6	5.9 (7.0)
2000	758	10	1.3	5.1 (3.9)
2001	582	14	2.4	6.1 (6.7)
2002	1,809	102	5.6	3.3 (1.8)
2003	444	7	1.6	4.4 (4.4)
2004	395	19	4.8	6.8 (5.2)
2005	1,767	40	2.3	5.0 (3.0)
2006	1,542	29	1.9	5.0 (3.5)
2007	1,458	40	2.7	5.4 (5.2)
2008	1,056	37	3.5	6.2 (5.2)
Total	12,989	407	3.1	4.8 (4.3)

Table 2. Recapture rates of monarchs in all five wing condition categories assigned in this project. Scores were such that a '5' represented wings that were fresh and undamaged and a '1' represented wings that were very worn and extremely damaged (see Fig. 1). Note that the total number of monarchs in this table (12,823) is lower than in Table 1 because 166 monarchs were not assigned a wing condition score.

Condition	# Captured	# Recaptured	% Recaptured
1	118	10	8.5
2	591	36	6.1
3	1,651	66	4.0
4	2,109	76	3.6
5	8,354	242	2.9

in wing lengths observed at this site will be examined in more detail in a subsequent manuscript. None of the two-way interaction effects were significant in the final model of wing length. Regarding the analysis of wing condition scores, we found that monarchs that were later recaptured had significantly lower condition scores (i.e. poorer condition) than those that were never recaptured ($F_{1,12780}=5.65$, $p<0.0001$; Fig. 2B), although the magnitude of this effect varied with gender (significant gender x capture interaction effect; $F_{1,12780}=7.87$, $p=0.005$). Again, other parameters in the model were significant, although not of primary importance for the current study; there was a main effect of gender ($F_{1,12780}=25.09$, $p<0.0001$) and year ($F_{12,12780}=2.05$, $p=0.017$) as well as significant gender x year ($F_{12,12780}=3.67$, $p<0.0001$) and year x capture ($F_{12,12780}=2.58$, $p=0.002$) interaction effects.

Finally, in the analyses of (log-transformed) stopover lengths (using all recaptured monarchs only), we found no significant effect of gender ($F_{1,380}=1.23$, $p=0.267$) on the number of days monarchs stayed at the site. However, there was an effect of year ($F_{12,380}=2.70$, $p=0.0017$). The annual variation in stopover lengths is readily seen in Table 1. The overall average length of stopover at this site was 4.8 days, but varied from 3.3 to 7.3 days. Importantly, there was a significant effect of wing size on stopover length ($F_{1,380}=6.69$, $p=0.0100$). Direct comparison of forewing length with (log) stopover length showed a weak negative relationship ($r=-0.11$, $p=0.0206$); smaller monarchs tended to have longer stopovers than larger ones. There was also a significant effect of wing condition on stopover length ($F_{1,380}=16.66$, $p<0.0001$), and this effect is evident in Figure 3, where average stopover lengths for each wing condition category are plotted. Monarchs with little or no wing

damage tended to have shorter stopovers than those with moderate to high wing damage.

DISCUSSION

One of the original goals of this study was to examine the characteristics of monarchs that stopover at this site in South Carolina and to determine if these individuals differ in any way from the general cohort that migrates through the site without stopping. In terms of wing size, stopover monarchs (those that were recaptured) did not differ from non-stopover monarchs; the initial decision to stopover or not is therefore not influenced by the size of the butterfly. However, of those that did stopover, smaller-winged individuals tended to remain longer at the site during stopovers, which is counter to the prior pattern where wing size did not influence recapture probability. The reason for this apparent discrepancy is not clear. It may be that size does not matter in terms of actual migratory flight behavior, but that for some reason, smaller individuals may require more time to forage and build their fat stores once on the ground. Regardless of the reason, if this phenomenon also occurs at other stopover sites along the entire migration pathway(s), one could expect the overall pace of migration to be somewhat slower for small monarchs than for large. This would be consistent with prior studies at other sites as well as our own, where large monarchs tend to predominate in the early phase of the migration period, with later monarchs tending to be small (Gibo & McCurdy, 1993; McCord & Davis, 2010).

Female monarchs appeared less likely to stopover at our site than did males (1.8% recapture rate for females vs. 4.3% for males). If this same pattern holds true for other locations in the migration pathway, it would suggest that females may differ from males in energetic requirements during migration (i.e. they need to stop less frequently than males). It is also not the case that females simply stay longer per given stopover bout (to compensate for fewer stopovers) since there was no gender difference in mean stopover lengths found here. This is consistent with data from another site in coastal Virginia (Davis & Garland, 2004). However, the original rationale for examining gender differences in stopover decisions was with respect to the disappearance of females from the migratory cohort (Davis & Rendon-Salinas, 2010). From the data gathered here, there is no evidence to support the idea that it is caused by females stopping more frequently or taking longer at stopovers than males. Prior analyses of data from this site also found no evidence for a gender bias in capturing

monarchs; females were far fewer in roost collections as well as in those collected while nectaring (McCord & Davis, 2010). Thus there is apparently still no clear explanation for the decline of females among overwintering monarchs over the last three decades. It may be that the explanation does not lie anywhere in the migration itself, but before the migration even commences; future studies may need to examine the possibility that females are becoming less likely to enter the migratory cohort to begin with. This idea may be plausible given that work with other butterfly species indicates males have a higher tendency to enter diapause than females (Wiklund *et al.*, 1992, Soderlind & Nylin, 2011).

Results from all three sets of analyses in this study indicate that the condition of a monarch's wings greatly influences its migratory stopover decisions. At this stopover site, wing condition predicted the likelihood of recapture, butterflies that were later recaptured had poorer wing condition on average than those that were not recaptured (Fig. 2B), and wing condition affected the length of stopovers; the greater the damage, the longer the stopover (Fig. 3). These results are consistent with our expectations that individuals with damaged wings are at a disadvantage in terms of flight ability (which is known in other insects; Combes *et al.*, 2010; Dukas & Dukas, 2011), and that such may translate into reduced foraging ability during stopovers, thereby yielding the need to spend more time obtaining food and turning it into fat stores (Brower *et al.*, 2006). Alternatively, monarchs with damaged wings may require more fat reserves throughout the migration if they burn more energy flying than do undamaged individuals, and thus would need to devote a greater amount of time foraging at stopover sites to meet this need. This would also account for the greater likelihood of recapture (of monarchs with poor wing condition); this indicates that such individuals choose to stopover more frequently than those with no wing damage or wear.

If our results regarding wing condition can be generalized across most stopover locations throughout the migration flyways, they may have more far-reaching implications for the role of migration in "weeding-out" suboptimal individuals. If monarchs with damaged wings stop more frequently during the migration and for longer periods at all stopover locations, their overall migration pace would eventually become slowed. In fact many of these individuals may never reach the overwintering sites in central Mexico. Indeed, this may explain why in collections of monarchs at overwintering sites, the majority of monarchs appear to have relatively

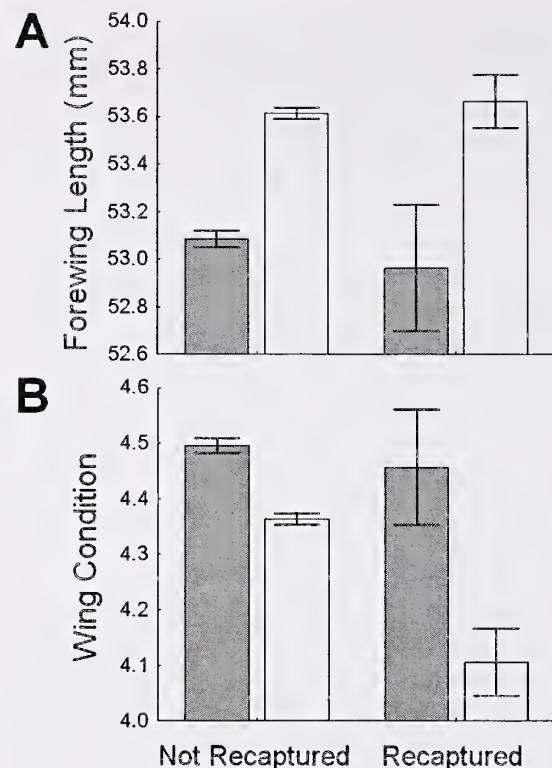


Figure 2. Average wing lengths (A) and wing condition scores (B) of monarchs that were never recaptured and that were later recaptured in the same season. Male (open columns) and female (grey columns) monarchs shown separately. Whiskers represent standard errors.

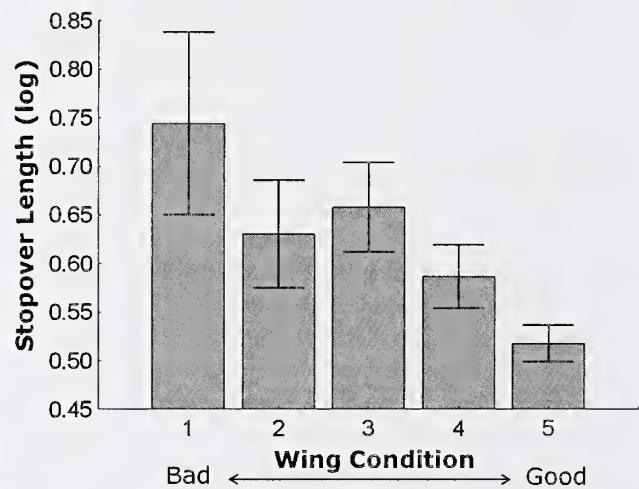


Figure 3. Average length of stopover (days, log-transformed) for monarchs assigned to each wing condition score in this project. Whiskers represent standard errors.

undamaged wings (A. K. Davis, unpubl. data).

There were certain aspects of stopover behavior at this site in South Carolina that are comparable to those found at a stopover site in coastal Virginia (Davis & Garland, 2004), despite there being only one year of data in that study (a later study at this site did not examine recaptures, Brindza *et al.*, 2008). First, the proportion of individuals that were later recaptured was nearly the same in both sites; out of 688 monarchs captured in one fall migration season at the Virginia site, 3.9% were recaptured, while at Folly Beach the overall rate was 3.1% across all years (although there was a degree of annual variation; Table 1). Second, the average length of stopover at Folly Beach was approximately 5 days. At the Virginia site it was approximately 3 days, using the same calculation for stopover length as the current study. These parameters (proportion recaptured and mean stopover length) should be easily obtained from other locations using similar methods, and in fact, we hope that future investigations of monarch stopover biology will strive to do this, especially in areas located in the central flyway which leads directly to the Mexican overwintering sites (Howard & Davis, 2009). This will allow for additional comparisons of stopover dynamics across a greater number of sites than is possible now. Moreover, this will also allow us to address more synthetic questions regarding monarch stopover, such as are monarchs more or less likely to stopover as the migration progresses southward, or are stopovers longer at locations near large water crossings?

Finally, we point out that this project serves as an excellent example of how monarch tagging can contribute to the scientific understanding of this species, as long as detailed records are kept and accurate data are recorded. Currently there are hundreds of individuals who tag many thousands of monarchs each year, but only for the purpose of having one of 'their' monarchs recovered in Mexico. As shown here, if records are kept on how many monarchs are recaptured at the site of tagging, these tagging activities would allow for a much greater breadth of questions to be addressed, and would broaden scientific understanding of the amazing migration of this species.

ACKNOWLEDGEMENTS

JWM is greatly appreciative of the support of many private property and home owners on Folly Beach (Island) who allowed him to capture monarchs. He also is thankful for the general support of both the South Carolina Department of Natural Resources, which provided monetary support for several monarch tagging seasons, and the Charleston County Parks and Recreation

Commission (CCPRC), which provided access to park property on the southwest end of Folly Beach (Island). JWM also received tagging assistance from CCPRC staff during several monarch tagging seasons. The manuscript was improved after helpful comments from Sören Nylin.

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Overnight perching aggregations of the aposematic Pipevine Swallowtail (*Battus philenor*: Lepidoptera: Papilionidae): implications for predation risk and warning signal use

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Abstract. Aposematic butterflies, those that are unpalatable and warningly colored, may aggregate during overnight perching to reduce the risk of predation. The conditions under which they aggregate and the postures assumed by perching butterflies may indicate how aggregations are a useful defense against predators, including the use of the warning signal. Additionally, studying these aggregations allows for a better understanding of the conditions under which their warning signal may be used. We investigated the overnight perching behavior of the aposematic Pipevine Swallowtail (*Battus philenor*) in both the field and in an enclosure. We found that the butterflies begin perching very close to sunset, when their blue iridescent warning coloration may still be effective, and the aggregations consist of between two and 21 individuals, which may accelerate warning signal learning by naïve predators. In both the field and enclosure, aggregated butterflies perched with the plane of their wings surfaces in parallel which suggests they perch in ways that increase the size of the warning signal. Additionally, *B. philenor* individuals perch in conspicuous locations which may facilitate warning signal detection, learning, and recognition. Our investigations of *B. philenor* aggregations lend support to the hypothesis that aposematic butterflies aggregate to increase the effectiveness of the warning signal against visually hunting predators.

Keywords: Warning coloration, aggregations, perching, *Battus philenor*

INTRODUCTION

Aggregations of aposematic animals, such as the overwintering and overnight aggregations of Monarch and *Heliconius* butterflies, are thought to provide enhanced protection against visually hunting predators (e.g. Turner, 1975; Sillén-Tullberg & Leimar, 1988; Gamberale & Tullberg, 1998). When aposematic butterflies aggregate, individual risk of predator attack can decrease through several mechanisms (Mappes & Alatalo, 1997; Gamberale & Tullberg, 1998; Lindström *et al.*, 1999). First, regardless of whether a predator's association of unpalatability with warning coloration is learned or innate, aggregations

can present a larger and, so, more effective warning signal (Gamberale & Tullberg, 1996a,b; Gamberale-Stille & Tullberg, 1999; Forsman & Merilaita, 1999). Second, aggregations may facilitate learning by naïve predators by 1) providing the opportunity for predators to see warningly colored individuals during or immediately following perception of distastefulness (Gagliardo & Guilford, 1993; Alatalo & Mappes, 1996), or 2) allowing predators to sample more prey in each encounter (Sillén-Tullberg & Leimar, 1988; Riipi *et al.*, 2001). By accelerating the learning process, fewer butterflies will be attacked and the individual risk for butterflies in the aggregation is reduced ("dilution effect"; e.g Bertram, 1978; Foster & Treherne, 1981). All these mechanisms rely on the predators seeing the butterflies and therefore may not be in force after dark for overnight aggregations.

Aggregations may also reduce the risk of attack by predators without the influence of the warning coloration. A naïve predator that attacks a group of aposematic butterflies may leave the aggregation after determining that prey are unpalatable (Alatalo & Mappes, 1996; Riipi *et al.*, 2001) and the risk of an individual being attacked is again reduced through the dilution effect. Predators will also be less likely

Received: 26 December 2011

Accepted: 27 January 2012

to encounter aggregated prey than solitary prey scattered throughout an area, because a finite amount of prey aggregated into larger groups will form fewer groups, decreasing the chance of encountering prey (Turner & Pitcher, 1986; Ioannou *et al.*, 2011).

With these potential benefits in mind and in order to better understand the conditions under which warning signals are used, we made observations on the dynamics and structure of overnight aggregations in the Pipevine Swallowtail butterfly, *Battus philenor* (Linnaeus, 1771). We suspected that *B. philenor* adults may aggregate because of their unpalatability, anecdotal reports of overnight aggregations (Scott, 1992; J. Fordyce and L. Gilbert, pers. comm.), and reports of feeding aggregations (Otis *et al.*, 2006). In March 2009, during a search for perching adult *B. philenor*, we observed overnight aggregations in the Mazatzal Mountains of Arizona, USA, and used this as an opportunity for further study of *B. philenor* overnight perching over two months. However, field observations were limited by access to the butterflies and so we expanded our observations and understanding of the aggregations by studying *B. philenor* perching behavior in an enclosure.

B. philenor is distasteful to predators due to the sequestration of aristolochic acids by the larvae (Sime *et al.*, 2000; Fordyce *et al.*, 2005). The ventral hindwing surface functions as a warning signal (Brower, 1958; Codella & Lederhouse, 1990) and displays both iridescent blue and orange spots (Fig. 1; Rutowski *et al.*, 2010). Both the iridescent blue and orange spots are recognized by predators as a warning signal and the most common predators of *B. philenor* in Arizona are insectivorous birds (Pegram *et al.*, unpublished observations).

We aimed to better understand how aggregations may reduce the risk of predation as well as the environmental conditions under which the warning coloration may be used by pursuing answers to four questions. First, do aggregations form and disband at times of day when visually hunting predators are active and when the warning signal is effective? If so, we expect that butterflies would aggregate before sunset or when ambient light is still available and disband after sunrise. Second, do aggregations form in locations that facilitate learning and recognition? To facilitate learning and recognition, we expect butterflies to perch in locations that make them conspicuous. Third, do butterflies position themselves in a way that increases the size of the warning signal? If so, we predict that the butterflies will orient themselves so that more wing surfaces are visible to an approaching predator. Finally, does the size of aggregations indicate that the butterflies aggregate to facilitate warning signal learning? If this



Figure 1. A *B. philenor* perched after sunset and illuminated with only indirect solar radiation. Even without the solar orb present in the sky, the blue iridescence of the ventral hindwing is visible.

is the case, we expect that there are more butterflies in aggregations than the number of butterflies required to be sampled by the predator for the predator to learn. Answers to these questions will help us to determine why these animals aggregate and how aggregations may influence the effectiveness of the warning signal.

MATERIALS AND METHODS

Field observations

We observed overnight aggregations of *B. philenor* from March to May 2009 at the confluence of Mesquite Wash and Sycamore Creek in the Mazatzal Mountains of Arizona, USA (N 33°43.784', W 111°30.997'; Fig. 2). Here, the riparian vegetation includes Sycamore (*Platanus wrightii*), Willow (*Salix* spp.), and Cottonwood (*Populus fremontii*) trees. The streamside area in which we made our observations was approximately 9000 m². On observation days, we arrived at the field site before sunrise or sunset and visually scanned trees with binoculars until we spotted *B. philenor* individuals.

Because some of the benefits of aggregation, such as increased signal size, can be realized with only two individuals, we considered two or more *B. philenor* butterflies perched together to be an aggregation. For each aggregation found, we determined how many individuals were clustered together (within a cubic



Figure 2. Riparian forest at the confluence of Sycamore Creek and Mesquite Wash in Arizona where aggregations of *B. philenor* were observed.

area of about 2 m on a side) and recorded the time at which the aggregation was first observed. On two mornings, we also recorded the time at which each individual left the aggregation. We obtained the sunrise or sunset time for each observation day from the NOAA calculator (<http://www.srrb.noaa.gov/highlights/sunrise/sunrise.html>), and compared all observed times to sunrise (for morning observations) or sunset (for evening observations).

We estimated height from the ground to the lowest butterfly for each aggregation using a known height as a reference. For 20 aggregations, we observed the orientation of each butterfly with binoculars and described it as the compass bearing of the azimuth of the line going from the wing tips to the body of the butterfly. This was always done before we observed any movement in the morning and after no more movement was observed in the evening.

To better understand how aggregations form and the activity around the time in which they perch, on four evenings we also counted individuals flying amongst the trees (at least 3 m above ground) every 15 minutes from a distant observation point that allowed us to observe the whole stand of trees. We stopped recording around sunset to observe the aggregations from a closer vantage point and take the above measures. Throughout the study, we also took notes on any interactions we observed among the butterflies.

Enclosure study

Due to limitations of the field study, in the summer of 2011 we also investigated the perching aggregations of *B. philenor* in a 10 m wide x 24 m long x 4.5 m high enclosure, the Maxine and Jonathan Marshall Butterfly Pavilion at the Desert Botanical Garden in Phoenix, AZ, USA. This enclosure is covered with 65% shade cloth and contains a large variety of vegetation and nectar sources, including Mexican Orchid trees (*Bauhinia mexicana*) and *Lantana* spp., but no hostplant. We populated the pavilion with lab-raised *B. philenor* that were either collected as eggs or larvae from the field site described above, or as eggs from females that mated in this pavilion and oviposited in the lab. Animals were raised to adulthood in an environmental chamber as described in Rutowski *et al.* (2010). We released individually marked *B. philenor* adults into the pavilion within 0-4 days of eclosion, and maintained a population of 6-20 individuals in the enclosure throughout the study. We always released butterflies at least two hours before sunset. The butterflies were an unstructured mix of males and females, and we recorded the sex of each before release.

To facilitate the assessment of the distribution of perched butterflies within the pavilion, we created a map of the interior of the enclosure, plotted on it the

location of perched individuals, and noted whether they perched in aggregations or individually. As with the field study, we defined an aggregation as two or more individuals perched within a cubic area of approximately 2 m on a side.

We measured the height of each perched individual with a tape measure. Also, as in the field we described the orientation of perched butterflies using the compass bearing of the azimuth of the line going from the wing tips to the body of the butterfly. These measurements in the enclosure are likely to be more accurate than those made in the field because we were able to more closely observe the butterflies.

We also focused on the formation and disbanding of aggregations. On five evenings, we plotted the location and recorded the height of every perched individual every five minutes, starting a half hour before sunset and ending a half hour after sunset. To understand how the aggregations disband, on five mornings, we recorded when each individual left the perch. We started this at sunrise and ended one hour after sunrise. In addition, we made qualitative observations on flight behavior and interactions among individuals forming aggregations at night or disbanding in the morning.

Statistical analysis

To determine whether perching individuals in the field and enclosure were oriented in a haphazard fashion we used circular statistics (Batschelet, 1981) using Oriana v.3 (Kovach Computing Services, Anglesey, Wales). We calculated: the mean angle; the Rayleigh statistic, which determines if the orientations are significantly different from random orientations; and the *V* test, which tests whether the butterflies were significantly clustered around specific compass bearings, with 180° and 0° as the given angles. We chose 180° and 0° as the given angles because we hypothesized that the butterflies may be perching with their wing surfaces perpendicular to the rays of the rising and setting sun. For the enclosure, we first sorted the orientation observations into those that were taken from aggregations and those that were taken from butterflies perched individually. We then calculated the mean orientation angle for each individual and ran the tests described above on these mean angles to control for multiple measurements on the same individual.

We determined whether height and propensity to aggregate were consistent among individuals using repeatability calculations. We calculated the repeatability (or *r*) and *p*-values (with a significance of 0.05) using one-way ANOVAs and the calculations described in Lessells and Boag (1987). To determine

whether individuals were consistent from day to day in their orientation, we used second-order circular statistics on the mean vector lengths, because linear statistics are not appropriate for angular measurements (Batschelet, 1981). We calculated the mean vector length for each individual using Oriana v. 3 (Kovach Computing Services, Anglesey, Wales) and then compared the distribution to the circular uniform distribution using the Kolmogorov's one-sample test (Batschelet, 1981).

The number of males and females in the enclosure on any given day was not equal. Therefore, to determine whether males and females perch in aggregations at the same rate, we used a *t*-test to compare the observed number of males in each aggregation to an expected number of males in each aggregation based on the sex ratio in the enclosure and the total number in the aggregation.

RESULTS

Field observations

We recorded data on 27 natural aggregations from 12 March – 5 May 2009 during 13 field visits (six in the early morning and seven around sunset). Nine of the aggregations were found at dusk and 18 were found at dawn. All aggregations were either found at the top or the outer edges of deciduous trees (Fig. 3). Heights ranged from 5.4 m – 10.6 m (mean = 7.9 m). Individuals started arriving at the site and flying around about 1 hour before sunset, and started to settle right around sunset. Counts of individuals in each aggregation ranged from 2-21 (mean = 5.8). Additionally, we found 10 individuals perched alone (10.5% of all butterflies observed), but our efforts in the field were focused on finding aggregations and so could easily have missed many solitary perchers. By 5 May, the trees had leafed out to an extent that made it difficult to scan for perched butterflies. We also found aggregations during future trips to the field site during other parts of the year when *B. philenor* was active (approximately March - October) suggesting that aggregations are not seasonal.

Aggregated butterflies measured in the field ($n=85$), were significantly oriented with the mean at 215° (Rayleigh: $z=13.9$, $p<0.001$; Fig. 4). We also did a *V*-test, which measures whether the observed orientations are clustered around a given angle. The *V*-test for 180° was significant ($p<0.001$) while the *V*-test for 0° was not ($p>0.999$), which means that the orientations of the butterflies were significantly clustered around 180°, that is their wings tended to point to the north.



Figure 3. An aggregation of six *B. philenor* high in a tree in the morning just before the animals disbanded. Note that three of the animals are dorsal basking.

Enclosure study

In June and July of 2011, we observed the overnight perching behavior of *B. philenor* in 38 visits to the enclosure on 33 different days, on some days visiting both in the morning and the evening. Our observations in the enclosure, as in the field, revealed individuals perching within aggregations as well as individuals perching alone (not within about 2 m of another butterfly). The mean percentage of individuals aggregating was 43% over all nights with a maximum of 65% on 21 July 11 and a minimum of 0% on 27 June 11 when only six individuals were present in the enclosure. The mean size of aggregations was 2.8, ranging from two to six individuals, and aggregations were composed of both males and females. The sex ratio of these aggregations was not biased toward either sex (t-test, $p = 0.464$).

Butterflies perched in aggregations ($n = 57$ individuals, 144 observations) were significantly oriented (Rayleigh test $z = 8.398$, $p < 0.001$) with a mean angle of 227.14° (Fig. 4). Also, as in the field, the orientations of aggregated individuals were significantly clustered around 180° (V-test, $v = 0.261$, $p = 0.003$) but not 0° ($v = -0.261$, $p = 0.997$), that is, with their wings pointed toward the north. Interestingly, butterflies perched individually (not in aggregations; $n = 57$ individuals, 173 observations) were not significantly oriented overall (Rayleigh test $z = 0.402$, $p = 0.669$; Fig. 4). In the enclosure, butterflies perched at heights ranging from 0.05–3.9 m (mean = 2.02 m), much lower than in the field and no doubt constrained by the height

of the pavilion's roof. As in the field, aggregations were found at the top or outer edges of trees and plants (Fig. 5), but were also found on the shade cloth and other structures within the pavilion.

In the enclosure, we could identify individuals and therefore determine repeatability or consistency in perching behavior among individuals. We found height ($r = 0.967$, $p < 0.001$) and whether they perched in aggregations or individually ($r = 0.814$, $p < 0.001$) to be consistent among individuals. However, orientation angle was not consistent among individuals (Kolmogorov's one-sample test; $T = 0.717$, $p = 0.762$).

We noticed that during their search flights in the evenings, individuals often landed on multiple perching spots before settling on a final perch between a half hour before sunset and a few minutes after sunset. Movements varied from slightly shifting their orientations to leaving for a new perching location up to several meters away. The mean number of times an individual landed on a perch before their final location was 3.1 (min = 0, max = 6). We also noticed that individuals already perching within an aggregation sometimes left after another butterfly arrived and flew around the perch, interacting with those already perched. Our observations ended about 45 minutes after sunset and, on 13 nights, we made observations the following mornings. On two occasions out of the 13, we found that the individual moved overnight and, on eight occasions, we were not able to find the individual anywhere in the pavilion and suspected they were attacked overnight. All of these individuals were perched alone. Predation may have been due to lizards (*Sceloporus* spp.) or roof rats (*Rattus rattus*), which were both spotted in the enclosure.

In the mornings, individuals opened their wings to bask, made small movements, or took off from their perches starting from a few minutes to one hour after sunrise. Most individuals moved to a different perching location after leaving their original night perch. We counted the number of perches until an individual started flying continuously or began feeding. The mean number of perches that individuals made after leaving their night perch was 1.4 (min = 0, max = 4). Aggregations disbanded one individual at a time, similar to how they formed. The shortest time from the first individual leaving to the last departure was 17 minutes for an aggregation of two individuals and the longest time from the first individual leaving to the last departure was 48 minutes for an aggregation of four individuals. However, we never observed any interactions between individuals within an aggregation during disbanding.

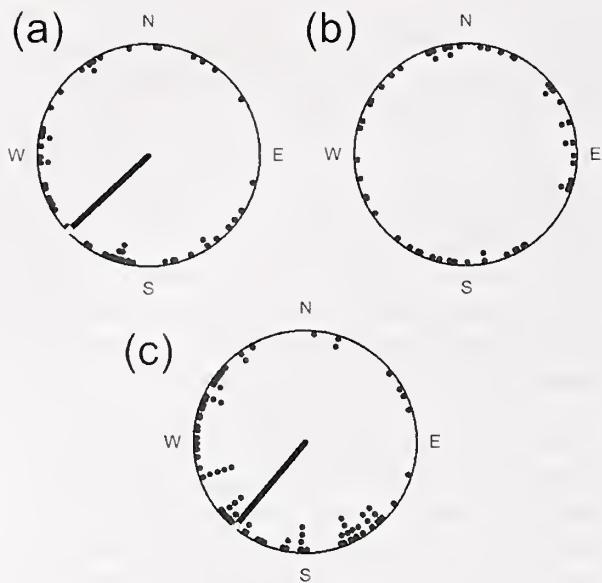


Figure 4. Orientation of a) aggregated butterflies in the enclosure, b) butterflies perched individually in the enclosure, and c) aggregated butterflies in the field. For the field observations, each dot represents one butterfly orientation, measured as the azimuth of the line going from the wing tips to the body. For the enclosure observations, these are averaged for each individual, so that each dot represents an individual. Butterflies in aggregations were significantly oriented (mean vector = 227° in (a) and 215° in (c)). The azimuths of the sunsets during measurement periods ranged from 270° to 299° and from 62° to 90° for sunrise.

DISCUSSION

In this study we set out to answer four questions about *B. philenor* aggregations and how they might influence predation rates: when and where the butterflies aggregate, the way the butterflies position themselves, and the size of the aggregations.

Do aggregations form and disband at times of day when visually hunting predators are active and when the warning signal is effective?

Insectivorous birds, the most common predators of *B. philenor* in Arizona, are active throughout the day, but may hunt more intensely around sunset or sunrise (e.g. Morton, 1967; Hutto, 1981). We found that *B. philenor* started perching around sunset but left their perches well after sunrise, which may indicate they are perching when their predators are most active.

The timing of the formation and disbanding of aggregations is likely to influence the effectiveness of warning coloration. The transmission and perception

of color signals are influenced by light environment (Endler, 1990; 1993). Under low light conditions, color signals become difficult to discriminate by birds (Cassey, 2009). Therefore, whether or not the solar orb is still present in the sky influences if and how predators learn or recognize the warning signal. The formation of aggregations around sunset or after the sun had set could limit the effectiveness of the visual signal. However, during field observations, the iridescent blue of the ventral hindwing was visible to the human eye even for some time after sunset but while there was still skylight (Fig. 1). This may be an advantage of displaying an iridescent warning signal. We also found that aggregations disbanded well after sunrise, so the warning coloration may be more effective at deterring insectivorous birds in the morning than in the evening.

Additionally, aggregating individuals may benefit from reduced predation through dilution or fewer predator encounters, as discussed earlier. Therefore, even though the aggregations are forming after sunset and diffusely reflecting warning colors may not be effective, iridescent warning colors may still be effective and aggregations may still reduce the risk of predation.

Do aggregations form in locations that facilitate learning and recognition?

A more conspicuous and larger signal may facilitate predator learning and recognition of a warning signal (Guilford, 1986; Gamberale & Tullberg, 1996b; Gamberale-Stille & Tullberg, 1999; Forsman & Merilaita, 1999; Gamberale-Stille, 2001; Prudic *et al.*, 2007). We found that *B. philenor* aggregations in the field average 5.8 individuals and form very high in trees. The area in which our observations took place is surrounded by mountains, and the sunshine clearly hits the tops of the trees first. This may allow for both the diffusely reflecting and iridescent warning colors to be effective earlier, as light becomes available to reflect off of the wings. Higher perching locations may also discourage predation by nocturnal, ground dwelling animals that may not be visually oriented and therefore not deterred by the warning coloration. In the enclosure, the average height of perching was only about 2 m off the ground but was likely constrained by the fact that the maximum height in the enclosure is only 4.5 m. We also found that aggregations were often formed on the outer edges of trees, which may also increase conspicuousness and, thus, warning signal effectiveness.

Despite perching in locations that may facilitate learning and recognition of warning signals through increased conspicuousness and signal size, microclimate could also be a factor driving *B. philenor* perch



Figure 5. An aggregation of four *B. philenor* in the enclosure taken in the evening.

selection and aggregations. Other butterfly species (e.g. *Danaus plexippus*) choose their perching location based on temperature and protection from wind and precipitation (Brower *et al.*, 2008; Salcedo, 2010). In *B. philenor*, perching high in trees may allow the butterflies to start basking earlier and therefore leave their perch, where they are most susceptible to predation (Rawlins & Lederhouse, 1978; Lederhouse *et al.*, 1987), earlier. An indication that *B. philenor* individuals are seeking specific conditions for perching is found in their evening activity. In the field and enclosure, we regularly observed interactions between perched and patrolling individuals in the trees during the evening. Individuals often settle on several perches before selecting their overnight perch. In the enclosure, most individuals landed on at least one perch before settling on a perch overnight. In the mornings, there is less interaction, but the butterflies still land on several perches before becoming fully active.

Do butterflies position themselves in a way that increases the size of the warning signal?

Butterfly orientation can have several implications for signaling behavior because the iridescent color on the wings will only be visible from certain angles and predators approaching on a path in the plane of the wing surface will not see any of the wing colors. We found that butterflies both within and among aggregations were similar in their body orientation in both the field and enclosure, but that non-aggregated butterflies were not. This suggests that butterflies may aggregate and position themselves to increase the size and, therefore, effectiveness of the warning signal. If

all of the butterflies in an aggregation are facing in the same direction, the warning signal they display is much larger to any potential predator approaching from a direction perpendicular to the plane of the wings and, in general, a larger warning signal is a more effective signal (Gamberale & Tullberg, 1996b; Gamberale-Stille & Tullberg, 1999; Forsman & Merilaita, 1999). An alternative hypothesis is that *B. philenor* butterflies could also be orienting themselves in order to increase the sun rays hitting the wings for warmth, but then we would expect to find that all perched butterflies significantly orient themselves to a direction perpendicular to the sun. This was not the case as butterflies perched individually were not significantly oriented.

Does the size of aggregations indicate that the butterflies aggregate to facilitate warning signal learning?

If a naïve predator is sampling prey from the aggregation and learning to avoid the animals based on the warning coloration, then the number of individual butterflies in the aggregation should increase with the number of prey the predator needs to sample to learn to avoid that prey item (Sillén-Tullberg & Leimar, 1988). For *B. philenor*, one experiment demonstrated that it takes an average of 2.67 butterflies for Blue Jays (*Cyanocitta cristata*) to learn not to attack this species using the ventral surface in a captive setting (Codella & Lederhouse, 1990). Considering the mean size of the observed aggregations was 5.8 for the field and 2.8 in the enclosure, predator sampling during learning could have influenced the size of *B. philenor* aggregations.

CONCLUSIONS

Our study provides information on the environmental conditions in which the warning signal of *B. philenor* is likely to mediate interactions between them and their predators and the ways in which by forming aggregations they may increase the effectiveness of their warning signal. We now know that *B. philenor* forms aggregations, selects postures within aggregations that may maximize the size of the warning signal, forms groups of a size that may facilitate predator learning, perches in locations that may facilitate learning and recognition, and forms aggregations at times during the day when iridescent warning coloration may be effective. Our observations revealed that the iridescence is still visible when the solar orb is not present in the sky, giving us a potential reason for why an iridescent warning signal might evolve. Our observation that the only

animals that disappeared from the pavilion overnight were individuals that were perched individually may also support the idea that *B. philenor* aggregations reduce predation risk.

ACKNOWLEDGEMENTS

We are grateful to the Desert Botanical Garden for use of their facilities and to Kamuda Pradhan and William Vann for help with field observations. This work was supported by The Lepidoptera Research Foundation, Inc. through their student research grant to KVP and the School of Life Sciences Undergraduate Research Program funded by the School of Life Sciences and the Howard Hughes Medical Institute. We thank Brett Seymour, Nikos Lessios, Melissa Lillo, Sophia Madril, Ellen Goh and Tyler Mello for comments on the manuscript.

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Catopsilia scylla (Linnaeus, 1763): A new record for Sri Lanka with notes on its biology, life history and distribution (Lepidoptera: Pieridae)

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Abstract. *Catopsilia scylla* was recorded for the first time in Sri Lanka in February 2008 and has been recorded since then in over 25 locations in the south-west quadrant of the island. Its larval food plant is *Senna surattensis* (Fabaceae: Caesalpinoideae), a widely planted introduced garden plant. The immature stages and behavior in Sri Lanka are documented here for the first time.

Keywords: Lepidoptera, immature stages, life history, larval food plants, Sri Lanka, Pieridae.

INTRODUCTION

Catopsilia scylla (Subfamily: Coliadinae; Tribe: Coliadini) comprises 10 subspecies that are widely distributed in southeast Asia and Australia from eastern Pakistan through Indo-China including Burma, the Malay Peninsula, Sumatra, Java, Lesser Sunda Islands, Borneo, Palawan, Philippines, Sulawesi Region, North & Central Maluccas, the Bismarcks, some of the Solomon Islands, northern Australia, New Caledonia and Fiji (Parsons, 1998; Vane-Wright & de Jong, 2003). It has recently been reported in Taiwan (Lu & Hsu, 2002). *C. scylla* is not found in India and has not been previously reported from Sri Lanka though *C. pomona* and *C. pyranthe* are common species there.

C. scylla was first identified in Sri Lanka by the authors on February 16, 2008 flying near *Senna surattensis* plants at km 58 on the A10 highway (the Kurunegala-Wariyapola road) in the North Western Province of Sri Lanka ($7^{\circ} 34' 60''$ N, $80^{\circ} 16' 60''$ E). Several adult males and females were seen flying near the plants and feeding on the nectar of flowers of *Caesalpinia pulcherrima* shrubs planted nearby. The *Senna surattensis* plants were laden with eggs, larvae in

all instars, pupae and pupal cases. Several specimens of *C. scylla* were also identified later that day in the authors' home garden (Bandarakoswatte, road B79 at km 44 about 12 km away from the original sighting). In fact, on February 4, 2008, the authors had noted in their field records "a very yellow *Catopsilia pomona*" [form pomona] at their property—very likely this was the first sighting of *C. scylla*.

A subsequent search over the next few weeks revealed adults and larvae at several other locations, most of them within 20 km of the original sighting. Other sightings ranged north to Padeniya, west to Pannala and Bingiriya, south-west to Rajagiriya and Colombo, south to Galle and Rumassala and south-east to Uduwalawe and Embilipitiya. Since then, it has been recorded at about 25 locations in the west, south, south-west and south-east of the island in most months of the year but is most common from January to April and September to December (Fig. 1).

Where information on the duration of developmental stages is given, these data were obtained in rearings at ambient temperatures (25–31°C) at Bandarakoswatte (07.37.01N, 80.10.57E), 70 m asl, North Western Province, Sri Lanka. Conventions used (applied to both the larva and the pupa): Segments are numbered S1 to S14 (S1—the head; S2 to S4—the 3 segments of the thorax; S5 to S14—the 10 segments of the abdomen).

Specimens (2 males and 2 females) have been deposited in the Sri Lanka National Museum.

RESULTS AND DISCUSSION

Adults: Wingspan 60–65 mm. Little or no variation

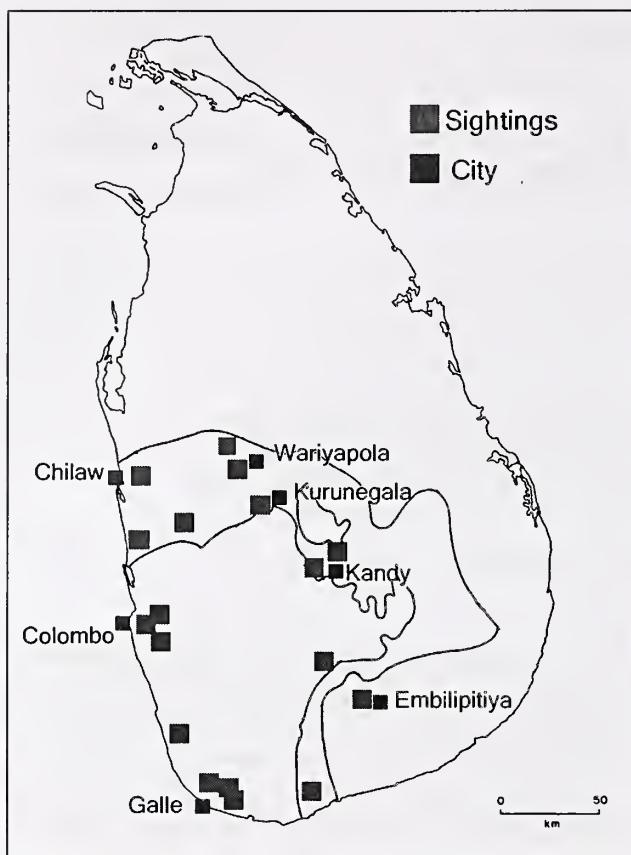


Figure 1. Distribution map of *C. scylla* in Sri Lanka.

has been observed in the adults. In the male the upperside of the hindwing is a cadmium-yellow without any orange; in the female the color is a less intense lighter yellow. There is no cell-end spot on the upperside of the forewing in the male (Fig. 2a) though there is a faint one in the female (Fig. 2b) which corresponds to the spot on the underside. The dark markings on the underside of the wings are purplish-brown (Fig. 2c). Though Braby (2004) reported a rare, pale, polymorphic form of the female in Australia, no such individuals have been reported in Sri Lanka up to now. Despite its distinctiveness in the hand, *C. scylla* may be superficially confused with *C. pomona* (form *pomona*) in flight. Nevertheless, it is distinctly smaller than *C. pomona* and has a slightly slower flight.

Subspecies: Specimens of *C. scylla* from Sri Lanka were compared to specimens in the NHM London, the Linnaean Society of London and MCZ at Harvard. The males of all 10 subspecies appear to be fairly similar though the color of the hindwing varies from orange to chrome-yellow; the females vary in the size and boldness of its markings, some

being heavily marked, others quite lightly marked. It has not been possible for us to determine by morphological appearance to which subspecies the specimens in Sri Lanka belong. Though most of the subspecies of *C. scylla* are variable in appearance, the individuals examined in Sri Lanka are quite invariable. The expression of the phenotype in Sri Lanka might differ from the parent stock from which it originated because of differences in photoperiod and temperature between the two locations. Nevertheless, the closest resemblances are to *C. s. scylla*, *C. s. etesia* and *C. s. cornelia*. Molecular studies are needed to determine its origin with certainty.

Behavior: The general behavior of *C. scylla* is similar to that of *C. pomona* but its flight is slower and it feeds more readily on flowers of herbaceous plants near the ground. Males are frequently seen near the larval food plant, presumably looking for females as they fly in and out of these small trees. Males have been observed mudsipping during dry weather. Females, as expected, were also observed mostly near the larval food plant seeking suitable oviposition sites. Both sexes feed on the nectar of flowers.

Female oviposition behavior: Eggs were laid singly on the lower or upper surface of a completely expanded leaf (Fig. 2d). Neither young shoots nor tender leaves, which are commonly used by *C. pomona* and *C. pyranthe*, were used for oviposition. The females deposited their eggs very rapidly and settled almost vertically whether they were laying on the upper- or under-side of the leaf. Most preferred to lay their eggs within 2 m of the ground and only a few oviposited above this height. Within a minute or two, they moved to other plants in the vicinity, deposited more eggs and moved on. Though some females were ovipositing on plants situated along a busy highway, they seemed unconcerned and were not driven away by the gusts of wind that swept through the trees as the large buses and trucks sped past on both sides of the road.

Courtship and adult nourishment: Courtship appears to be absent. On March 2, 2008, the authors observed a female being mated as soon as it emerged from the pupa, with no apparent choice by the female. On another occasion, a female who had just flown from its pupal case was intercepted immediately by a male flying by and mated; both settled down on a herbaceous plant near the ground. On one occasion, a mated pair remained in copula for 1 hour, 32 minutes—a time period that seems very long, although this may not be usual.

Adults feed on the nectar of a number of common plants. Some are naturalized plants that are a favorite nectar source for many species of butterflies—these



Figure 2. Adult *C. scylla* butterflies from Sri Lanka. **a.** Male, upperside. **b.** Female, upperside. **c.** Undersides, female on the left, male on the right. **d.** Female ovipositing on *Senna surattensis*.

include *Stachytarpheta indica*, *Chromolaena odorata* and *Cordia curassavica*. Others are not naturalized but are widely planted—these include *Duranta repens*, *Pseuderanthemum latifolium*, *Zinnia*spp. and *Caesalpinia pulcherrima* (a favorite of *C. scylla*, perhaps in part because it is often planted beside *S. surattensis* on the roadsides). Still others, such as *Urena lobata* and several species of *Sida*, are native. This propensity to feed on the nectar of a wide range of common plants has probably contributed to its rapid expansion and colonization of new areas.

Members of the genus *Catopsilia* are reported to be migrants in many different countries including India (*C. pomona* and *C. pyranthe*, Larsen, 1978), Australia (*C. pomona*, Braby, 2000) and Africa (*C. florella*, Larsen, 1992). In Sri Lanka, *C. pomona* and *C. pyranthe* have been recorded migrating twice a year starting near the beginning of each monsoon season (i.e. February-April & October-December) (Williams, 1927). Manders (1904) reported the direction of

flight of *C. pyranthe* and *C. pomona* as being south from Trincomalee down along the east coast, west along the southern coast and north up the west coast. He further reported that *Catopsilia* butterflies all began to migrate at the same time, regardless of what part of the country they lived in. He found that the majority of the specimens in the October to December flights were females who were desperate to lay their eggs and laid them indiscriminately on any bush. The authors have noted *C. pyranthe* and *C. pomona* migrating to the south from their home location at Bandarakoswatte in April and October. There are no reports of *C. scylla* being migratory though Braby (2000) speculates that it is almost certainly migratory in Australia. But in Sri Lanka, *C. scylla* appears to engage in migratory behavior. When they were first identified in 2008, they appeared to be moving in numbers towards the south and south-east. Further observations seemed to show that it disperses widely but not in any particular direction. Adults have a strong tendency to fly along

roadways perhaps because its larval food plant, *Senna surattensis*, is frequently planted along these roads.

Immature stages: The final instar larva and pupa were briefly described by de Nicéville & Martin (1895) (quoted in Parsons, 1998): the larva is “dark velvety green, with a yellowish-white lateral stripe, and ... minute black dots on the upper edge of this stripe are most dense on the thoracic segments” and “the pupa is similar to that of *C. pomona* but [slightly] shorter and more convex (i.e. less slender than that of *pomona*).” These brief descriptions agree with the investigations of this study. In addition, we have recorded the following:

Egg: 1 mm × 0.3 mm, white, spindle-shaped, more slender and stalk slightly narrower than in *C. pomona* (Fig. 3a). 1st instar: completely white on emergence, next day turned pale yellowish-green with a pale yellow head (Fig. 3b), ate tissue on the underside of the leaf on either side of the midrib leaving the upper epidermis intact which later dries up to leave a small hole in the leaf that gives away the presence of the larva (Fig. 3c), ate old leaves as well as young ones, rested along the midrib of the leaf on the underside, movement somewhat looper-like. 2nd instar: head light green with small black tubercles, body paler green, rugose, each segment with 4-6 furrows, each furrow with numerous small black protuberances, each protuberance with several fine setae, indistinct spiracular line, ate tissue from the margins of the leaf, rested along the upperside of the midrib of the leaf or along the leaf rachis, fed mostly at night (Figs. 3d). 3rd instar: same as 2nd but with a faint white spiracular line (Fig. 3e). 4th instar: same as 3rd instar, white spiracular line (sometimes faint) with a tinge of yellow on its upper margin, larger black spots just above spiracular line (Fig 3f, g). 5th instar: head same color as body or slightly lighter or yellowish, abdomen green to greenish-yellow, spiracular line cream to white, sometimes with a tinge of bright yellow on its upper margin; each segment transversely ridged (4-6 ridges per segment), each ridge with 20-30 small raised black protuberances on each of which are found several black setae, size of the protuberances variable but those just above the spiracular line are the largest within each ridge though they may be absent toward the last few segments, those on S3-S5 are the largest (Figs. 3h-j). The larva is very similar to that of *C. pomona* but can be distinguished by the black protuberances above the spiracular line. In *C. pomona*, they are large and prominent along the whole length of the abdomen while in *C. scylla*, they are most prominent in the thoracic region and absent or not prominent on the other segments. In all instars, the molted cast-off skin is often eaten by the larva. The

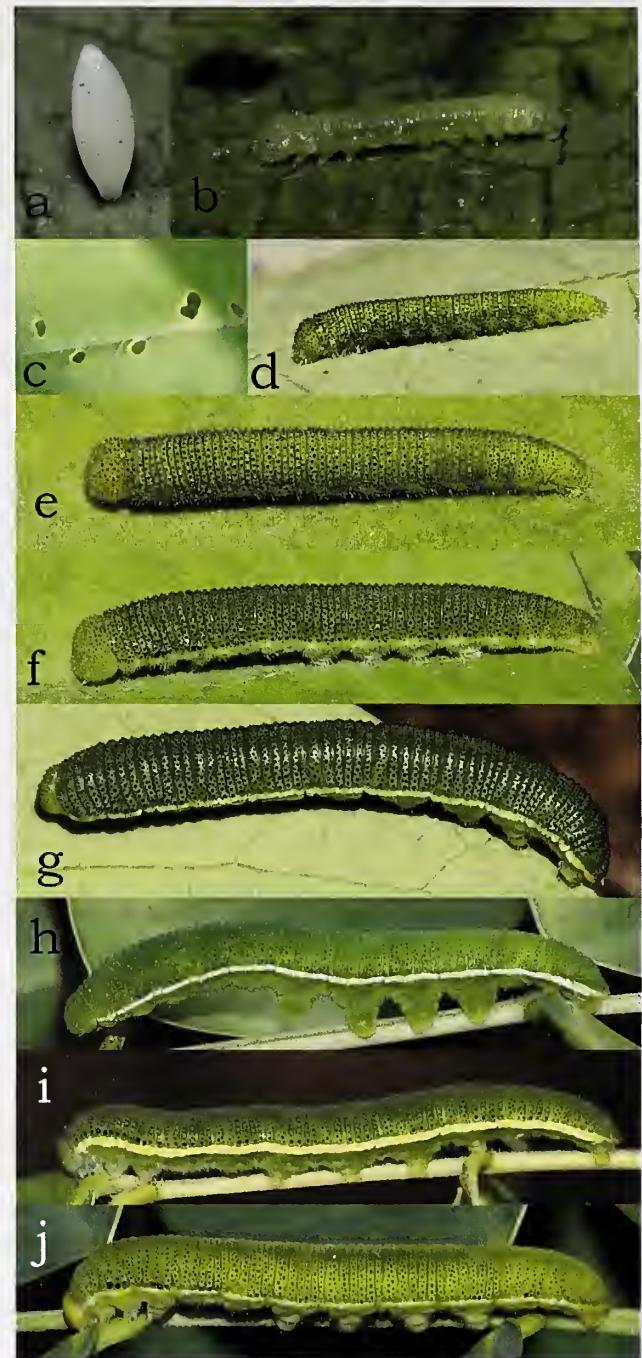


Figure 3. Early stages of *C. scylla* from Sri Lanka. **a.** Egg. **b.** Larva, first instar, one day after emergence. **c.** Holes in leaf left by first instar larva after eating. **d.** Larva, second instar, one day after molt. **e.** Larva, third instar. **f.** Larva, fourth instar, with faint, interrupted spiracular line. **g.** Larva, fourth instar, with complete white spiracular line. **h.** Larva, fifth instar, green head and body with few black supraspiracular spots. **i.** Larva, fifth instar, green head, yellowish-green body with numerous black supraspiracular spots. **j.** Larva, fifth instar, yellow-green head, yellowish-green body.

first two instars fling their frass. The larva frequently rests with the upper half of its body raised and is quite unresponsive to disturbances, hardly moving when touched. The larvae seem to be quite tolerant of an unclean environment: those found on *S. surattensis* plants along the roadways fed readily and survived well on leaves that were covered with a thin layer of soot and dust. Pupa: with a faint white lateral line from S1–S7, more sharply defined white line from S8–S13 and yellow on S14 (in *C. pomona*, this line is completely yellow), horn light yellow at tip (in *C. pomona* with a black dot), S4–S8 and S9–S14 more convex particularly on the ventral side, spiracles white; color ranges from green to yellow (Figs. 4a, b); pupa loosely fastened to the substrate (Fig. 4c).

Length of larva (mm): on emergence (4); after 3rd molt (16); at maturity (32–43); pupa (26–30). Duration of immature stages (days): Egg (1–3); 1st instar (1–3); 2nd (2–4); 3rd (1–3); 4th (3–4); 5th (4–5); pupation (1); pupa (7–11); egg–adult (24–32).

Larval food plants: In Sri Lanka, the only confirmed larval food plant is *Senna surattensis* (Fabaceae) which is an introduced small tree. It is widely planted but does not yet appear to have become naturalized. It produces large amounts of viable seeds but because the pods remain attached to the plant and do not dehisce, the seeds do not fall to the ground to germinate and eventually rot on the tree (Fig. 4d). Since *S. surattensis* is planted as an ornamental, it is restricted to urban and semi-urban landscapes. *C. scylla* is found only in such habitats and has not yet established itself on other plants in forest settings unlike *C. pomona* and *C. pyranthe*.

Larval food plants used by *C. scylla* have been recorded for several other countries. For the Sulawesi area, Vane-Wright & de Jong (2003) reported *Crataeva* (Capparaceae), *Cassia*, *Senna* and *Tephrosia* (Fabaceae). In Australia, Braby (2000) reported *Senna leptoclada*, *S. retusa* and *S. surattensis* (an introduced plant) while Wells and Houston (2001) also included *Senna didymobotrya* (introduced). In Papua New Guinea, Parsons (1998) reported that *Cassia spectabilis* [now *Senna spectabilis*] and *C. tora* [now *S. tora*] were used. Corbet & Pendlebury (1992) reported *Cassia fistula* and *C. obtusifolia* [now *Senna obtusifolia*] from Java, and that “larvae were found on *Tephrosia candida*” in Malaya. In Singapore, where *C. scylla* is a common urban butterfly, *Cassia fistula*, *C. biflora* and *C. tora* are used.

There are 30 species of *Senna* and *Cassia* in Sri Lanka (Dassanayake, 1991) including many that are used as larval food plants in other countries. It is possible that *C. scylla* may utilize some of these as

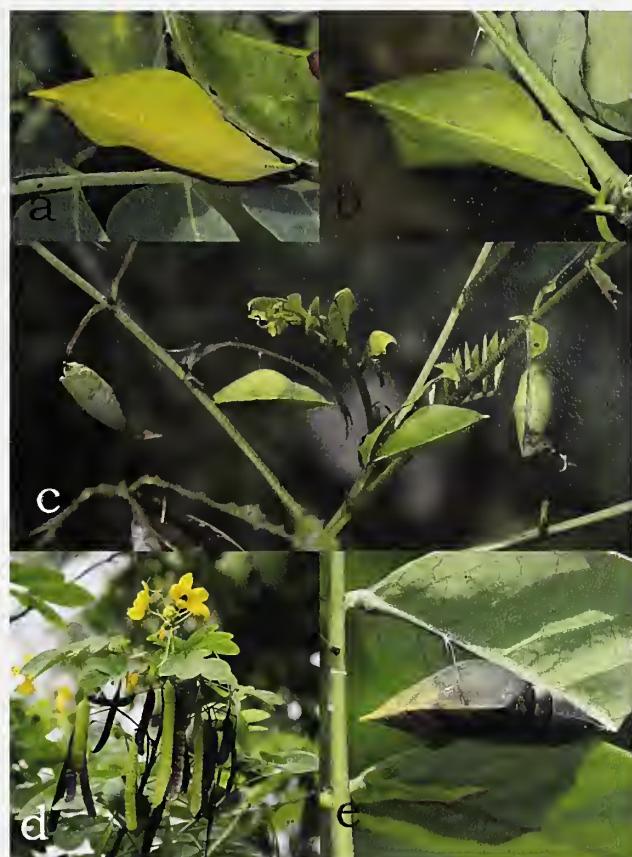


Figure 4. Pupa and host plant of *C. scylla* in Sri Lanka.
 a. Pupa, yellow form. b. Pupa, green form. c. Pupae loosely connected to substrate. d. *Senna surattensis*, flowers and pods. e. Pupa with fungal infection.

larval food plants in the future. In 2010 (February 15–20), a female was observed laying eggs on *Cassia auriculata*, a small tree native to Sri Lanka, called “ranawara” in Sinhalese. Eggs were collected with difficulty as they were laid high up, but the larvae all died within a few days of hatching out though they ate the leaves readily. Other eggs that had been laid by *C. pyranthe* on the same plant hatched out and were raised successfully. It is possible that some larvae of *C. scylla* might be able to survive on *C. auriculata* with the passage of time.

Possible origin: It is not clear how long *C. scylla* has been in Sri Lanka. Though several workers were very active in butterfly studies from about 1870 to 1950, there have been few people doing research on butterflies since then. The authors' own studies from 1960 to 2004 were sporadic and of short duration and the authors took only short occasional trips to the area where *C. scylla* seems to be most concentrated. It is possible that *C. scylla* was overlooked. However, it is

distinctive enough for a butterfly enthusiast to have spotted it if it was present in Sri Lanka earlier.

Though *S. surattensis* is an introduced plant, it has been recorded on the island since at least 1824 when it was listed by Moon (1824) in his catalogue as *Cassia glauca*. Moon reported it being found in Colombo and with the Sinhalese name of wal-ehela that is still in use today though it is now more commonly called “Malaysian ehela.” The name wal-ehela is also applied to *Senna bacillaris*. Thwaites (1864) listed it with the same information as Moon. Trimen (1894) listed it saying that he knew it only as a “garden plant”. Willis (1911) listed it but with no other information. Dassanayake (1991) listed it as an introduced ornamental but gives only a few locations. The Forest Department now supplies plants grown from local seeds as a horticultural plant and it is planted widely in home gardens. Its distribution is in the south-west quadrant of the island.

In the 1980’s large specimens of *S. surattensis* were imported from the far east (probably Malaysia or Singapore) and planted extensively along major roadways including the highway A10. It is probable that *C. scylla* arrived here as eggs, larvae or pupae on this imported plant material and escaped detection as it passed through quarantine. It is highly unlikely that *C. scylla* flew across the sea because of the large distances involved; the closest populations are in Malaysia and Thailand and *C. scylla* would then likely have arrived on the east coast from which we have no records. It is also possible that adults came across on merchant vessels but there is no evidence yet to support this hypothesis.

Prospects for the future: *C. scylla* is likely to remain well-established in Sri Lanka because its larval food plant is widely distributed and is a popular, hardy garden plant. Since *C. scylla* feeds on a variety of plants in other countries, it is possible that it might cross over to feed on other species that are found in Sri Lanka such as *Senna didymobotrys*, *Cassia tora*, *C. spectabilis*, *C. fistula*, *C. obtusifolia* and *Tephrosia candida*.

Catopsilia species are noted for their ability to disperse widely and in great numbers and for their fluctuations in populations (Larsen, 1992). It is likely that the population structure of *C. scylla* will be similar to that of *C. pomona* with peak populations coinciding with the intermonsoonal rains (March/April and Sept/Oct). However, given its predisposition to lay eggs on older leaves and the ability of the larva to feed on them, *C. scylla* may have different flight periods and population peaks. The frequent flushing of *S. surattensis* throughout the year may also influence the population dynamics of the species as edible food material is available all year. We do not yet have

enough data to quantify these observations.

However as *C. scylla* becomes more established, it will tend to become more susceptible to parasites and pathogens. Larvae collected in February 2008 all survived with no problems though a few pupae appeared to be infected with a fungus (Fig. 4e). In February 2010, many of the larvae that were collected died in the larval stage possibly due to a fungal infection, but in 2011-2012, larvae again were numerous and healthy. In 2008, *S. surattensis* trees were literally loaded with eggs, larvae and pupae at the same time. Now, only a few individuals (eggs or larvae) are seen on an individual plant. The most recent records, from December 2011, showed several eggs on only one tree out of 10 that were checked though in another area a few kilometers away, a single tree had several eggs and more than two dozen larvae of various instars.

S. surattensis is also used extensively by *Eurema blanda silhetana* as a larval food plant. *E. blanda silhetana* larvae are gregarious and so offer some competition to *C. scylla* though they feed only on young leaves unlike *C. scylla*. In the course of this study, a new larval food plant record was ascertained for *C. pomona*: its larvae were also found to feed on *S. surattensis*. *C. pomona* also feeds on older leaves and so may offer some competition. In addition, red tree ants (*Oecophylla smaragdina*), which prey on almost any living creature including butterfly larvae and pupae, often build their nests on *S. surattensis* plants. Female *C. scylla* carefully check the trees before laying their eggs and avoid laying on plants that harbor these ants.

CONCLUSION

Larsen (pers. comm.) and Pittaway *et al.* (2006) noted that “firm establishment of exotic butterflies is a very rare event. There are not more than 20–30 similar cases – and there are more than 18,600 recorded butterfly species worldwide (Larsen, 2005)”. Though its numbers seem to have declined since the first sightings 4 years ago, this fits with the population characteristics of many species of *Catopsilia* that go through cycles of rapid expansion and then contraction. Though the data is somewhat scanty because of the lack of observers, *C. scylla* seems to be well-established in Sri Lanka with a good supply of larval food plants and a niche for its larvae. As we look through our window, we still see them flying.

Faunistic data

All records by the authors unless otherwise noted (ordered by location): Road A10, km 44 (5 ii 2010); road A10, km 58 (2008: 16

ii, 23 ii, 25-26 ii, 29 ii, 2 iii, 18 iii, 5 iv, 24 iv, 2 v, 3 vii, 22 ix, 24 ix, 2 xi, 20 xi, 3 xii. 2009: 17 i, 30 i, 18 ii, 9 x, 2 xii. 2010: 18 ii. 2011: 20 ii, 29 vi, 7 xii, 22 xii. 2012: 4 i, 10 i); Bandarakoswatte, road B79, coconut property at km 44 (2008: 4 ii, 18 ii, 25 ii, 6 iii, 12 iii, 7 iv, 8 iv, 21 iv, 12 vii, 30 ix, 7 x, 13 x, 5 xi, 10 xii, 15 xii, 22 xii, 29 xii. 2009: 7 i, 17 i, 26 i, 4 ii, 12 ii, 17 ii, 4 iii, 12 iii, 22 iii, 28 iii, 12 iv, 16 xi, 25 xi, 12 xii, 15 xii, 16 xii, 30 xii. 2010: 12 i, 14-15 i, 20 i, 15 ii, 3 iii, 16 iii. 2011: 24 i, 11 ii, 1 iii, 10 iii, 15 vi, 8 vii; 10 xii, 15 xii. 2012: 3 i, 8-10 i); Padeniya, road A10 (4 xii 2011); road A26, km 6 (9 vi 2009); road B79, km 17 (28 ii 2008); road B79, km 47 (6 iii 2008); road B79, km 53 (23 ii 2008); road B79, km 48 (2008: 22 v, 24 v, 19 vi, 2 xi, 20 xi. 2009: 3 xii); Balangoda/Rassagala Road (1 ix 2010); Bingiriya (14 ii 2010, A. Amarakoon, pers. comm.); Colombo (25 ix 2010, C. de Alwis, pers. comm.); Digana Kandy (24 ii 2009); Dolukanda Kurunegala (8 xi 2008, A. Amarakoon, pers. comm.); Embilipitiya (8 v 2008, D. Ranasinghe, pers. comm.); Galle (1 xi 2009, N. Thotawata, pers. comm.); Ganewatte Kurunegala (9 xii 2008); Hiyare Kuruduwatte Galle (1 xi 2009); Kandy (1 xi 2009, N. Thotawata, pers. comm., 26 xii 2009, A. Amarakoon, pers. comm.); Kochikade (31 x 2008); Koggala & Kottawa, Galle (– 2008, Galle Biodiversity Survey); Pilassa near Kurunegala (23 iii 2008); road A10 at Mallawapitiya (2008: 20 ii, 1 iv); Nadugala Matara (– 2009, Matara Biodiversity Survey); Open University Nawala (20 x 2009); Padeniya (20 ii 2008, 30 i 2009); Pannala (10 v 2008); Pannipitiya Colombo (5 xii 2008); Peradeniya (2010: 18 ii, 27 ii); Rajagiriya Colombo (2008: 4 iii, 28 iii, 30 v); Rumassala Galle (8 v 2008); Sri Jayawardenapura, Colombo (31 x 2008); Talangama Lake, Colombo (31 x 2008); Udawalawe (– 2009, D. Ranasinghe, pers. comm.); Wakwella Galle (– 2008, Galle Biodiversity Survey).

Dates of breeding (oviposition, eggs, larva or pupae) were noted in the following months/years): at Bandarakoswatte—2008 (Feb, Mar, Apr, Jul, Dec); 2009 (Feb, Jun); 2010 (Feb); 2011 (Feb, Mar, Jun, Jul, Dec); 2012 (Jan); at Embilipitiya—May 2008.

ACKNOWLEDGEMENTS

We thank the following: Michael Braby; Blanca Huertas of NHM London, Mike Fitton at the Linnaean Society London, and Philip Perkins and Stefan Cover at MCZ Harvard for assistance with specimens; Department of Wildlife Conservation, Department of Forestry and the Biodiversity Secretariat of Sri Lanka for their support of this research.

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BOOK REVIEW

***Owlet caterpillars of Eastern North America* by D. L. Wagner, D. F. Schweitzer, J. B. Sullivan & R. C. Reardon, 2011**

Princeton University Press, Princeton & Oxford, 576 pp. ISBN 978-0-691-15042-0. Price: appr. US\$ 35.00.

Caterpillars of Macrolepidoptera are generally well known with regard to their morphology, diversity of resource affiliations, and ecology. Strikingly enough, however, the identification of caterpillars found in the field still often confronts the researcher with a severe challenge. Even for well-studied regions such as Europe, Japan or North America, few books are available which allow for the quick, easy and safe identification of caterpillar samples beyond the few commonest species. Available treatments tend to be either very 'technical' in style, or if more 'popular' in approach they are extremely incomplete in their species coverage. Or, in some cases, good illustrations are embedded in large multi-volume monographs which are not really convenient to use for identification purposes. Yet, this is what ecologists, conservationists, or dedicated amateurs would demand the most. The present volume fills this gap for the owlet moths (larger part of the superfamily Noctuoidea) from eastern North America, and it does so in a remarkable manner. More than 800 species are covered from the families Noctuidae and Erebidae, following the modern concepts of phylogeny and systematics of the Noctuoidea. Two erebid subfamilies (Arctiinae and Lymantriinae) are not included since they have been dealt with in an earlier companion volume (Wagner, 2005), with 44 and 10 species, respectively. Also Notodontidae (50 spp.) were already treated in that earlier volume, such that color figures are now available for distinctly over 900 species of North American Noctuoidea in just two books.

The new book starts with a concise, but

scientifically up-to-date introduction into caterpillars: their role in ecology, their morphology and diet, techniques for sampling caterpillars in the field or raising them from captured adults, and classification. The heart of the book is the species accounts, sorted according to the newest systematics. For each subfamily first a general introduction is given, followed by detailed treatments of species. In most cases, each species has its own page, with superb color photographs of a mature larva (but often accompanied by smaller pictures of other instars, or different color morphs) plus color shots of living and spread adult moths. Each species account is densely packed with valuable information on diagnostic characters, habitats, host plants and other details about bionomics, behavior, or life-history. In addition to these full accounts, a sizeable number of species is portrayed in more condensed manner, usually 2 to 4 species per page. A glossary, an extensive references list, and two separate indexes to host plants and moths supplement this book.

This volume is really a 'must-have' for any lepidopterist or ecologist with interest in caterpillar biology. Not only are the illustrations of stunning quality. Also the scientific content meets almost all what one might wish to learn from such a book, at least as an ecologist or caterpillar enthusiast. The text avoids burdens of technical jargon and the book remains concise in its approach. In this regard, Wagner *et al.* differ distinctly from the 3-volume monumental monograph by Ahola & Silvonen (2005-2011) on North European owlet moths. This latter bilingual series provides far more extensive anatomical details in a more 'scientific' style and also includes identification keys. For the systematic specialist with particular interest in the Noctuoidea, this type of monograph series is of course essential. But the quality of color illustrations does not meet what Wagner *et al.* now have produced for a significant complementary fraction of the Holarctic noctuid fauna. Remarkably, the novel book by Wagner *et al.* is also offered at a very modest price. In sum, the *Owlet caterpillars of Eastern North America*

Received: 16 March 2012

are a most accessible source of information for any naturalist. If you are going for a first and intense encounter with the realm of Nearctic owlet moth larvae, their fascinating morphological and ecological diversity, the book by Wagner and colleagues is the way to go. It would be great to see similar volumes being published on the remaining large 'macro-moth' family Geometridae from the Eastern United States, or companion volumes for the Western part of the continent.

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The correspondence between John Gerould and William Hovanitz and the evolution of the *Colias* hybridization problem (Lepidoptera: Pieridae)

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Abstract. The recently-discovered correspondence between Prof. John Gerould of Dartmouth College, who initiated the study of hybridization between sympatric North American species of *Colias*, and William Hovanitz, who made it the centerpiece of his research program in the 1940s and 50s, sheds light on the sources of Hovanitz's ideas and the evolving interpretation of that system, which remains a major challenge at the interface of population ecology and population genetics today.

Keywords: John Gerould, William Hovanitz, *Colias eurytheme*, *Colias eriphyle*, *Colias philodice*, interspecific hybridization, hybrid zone theory.

INTRODUCTION

Hybridization between sympatric North American species of the genus *Colias* Fabricius (Pieridae) remains one of the most vexing problems in the evolutionary ecology of butterflies and, more broadly, in our understanding of interspecific hybridization as a phenomenon. Hybridization between *C. eurytheme* Boisduval and *C. philodice* Godart *sensu lato* (now generally separated into two species: *C. philodice* in the East, and *C. eriphyle* Edwards in the West) has been studied since the 19th century in the West and since the advent of sympatry in the Mid-Atlantic States in the 1920s. Despite a very large bibliography (mostly cited in Jahner *et al.*, 2012), the phenomenon remains poorly understood. Basically, the question is as follows: wherever *C. eurytheme* is sympatric with one of the others, they hybridize, often at high frequency, yet they retain their separate identities and do not fuse. The "hybrid zone" includes virtually all of the continental United States except peninsular Florida and California west of the Sierra Nevada;

and parts of southern Canada. Jahner *et al.* (2012) recently reviewed the situation historically and demonstrated, using path analysis, what factors seem to be the primary drivers of hybridization frequency at one locality which was sampled for 66 consecutive generations. They did not, however, identify the factor or factors keeping the populations distinct. Jahner *et al.* provide (partly as an Appendix) an historical retrospective on the study of hybridization in North American *Colias*, which is too large and complex a subject to reprise here. Both their paper and the Appendix can be obtained by e-mailing the author of this paper.

The two researchers who contributed the most to our understanding of *Colias* hybridization during the 20th century were Professor John Gerould of Dartmouth University and William Hovanitz. From their published works it is clear that Hovanitz derived inspiration from Gerould, but until a large, if incomplete, collection of their correspondence surfaced in 1993, it had not been known how frequent and detailed – and often contentious – their interchanges had been. Gerould's papers were deposited at Dartmouth after his death, and his library was sold off. Somehow the Hovanitz correspondence traveled with the library and ended up in the hands of an antiquarian bookseller, from whom I purchased it on December 9, 1993. The entire file will be donated to the Gerould archive at Dartmouth with the completion of this article. The letters, mostly originals from Hovanitz and saved carbon copies of Gerould's, begin in 1939 and continue through 1950. During the early part of the correspondence Hovanitz

Received: 29 March 2012

Accepted: 13 April 2012

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was a doctoral student at the California Institute of Technology under Nobel laureate Thomas Hunt Morgan and the also-distinguished A.H. Sturtevant. From 1943-1945 he had various assignments, largely focused on medical entomology, in South America, Michigan and Florida. He then studied with the ecologist Lee Dice at the University of Michigan before joining the faculty of Wayne State University in Detroit. His earliest *Colias* papers were based on research conducted as a graduate student and give his institutional affiliation as Cal Tech. Later productions emanated from Dice's Laboratory of Vertebrate Zoology at Ann Arbor and from Wayne. Miller (1979) published a bibliography of Hovanitz's publications.

THE EARLY CAL TECH PERIOD

Hovanitz's first letter to Gerould, dated September 17, 1939 – at the start of his first term in residence at the William G. Kerckhoff Laboratories in Pasadena – is of a sort very familiar to academics. It begins:

Dear Prof. Gerould:

For some time I have been interested in the variation of color in the scales of butterfly wings—especially as regards the relationship between ecologic habitat and the type of variation. Although as yet, I have not had the facilities for extensive experimentation, I hope in time (perhaps starting this winter with desert races) to be able to work on the physiology and genetics of color in the Nymphalidae...

He then sketches out a potential research program which would eventually be partially realized in his studies of parallel color variation in *Melitaeini* along climatic gradients. But he faces obstacles:

I would like to break the [larval] diapause and as I have found little in the literature of help, I wonder if you can help me with ideas? A second problem comes with mating. Though I have bred many insects, I have never tried to mate butterflies in captivity. Do you know any special way of accomplishing this?

In a handwritten PS, he asks for any available reprints on "butterfly genetics, etc." There is no mention of *Colias*. Clearly, Hovanitz did not have *Colias* in mind when he initiated contact. Presumably Gerould sent him reprints of his important *Colias* papers, which may account for the change in the thrust of Hovanitz's research which is evident a year later. Unfortunately, the beginning of his interest in *Colias* is lost. The next item in the file is from Hovanitz, dated Sept.10, 1940; by now he is deeply into *Colias* work, both in the field and the lab:

I wonder if you could get or know of someone who could get me some living material of *Colias philodice*. I am now breeding

eurytheme and some of its Great Basin varieties which are intermediate between the two....

I am working on a curious population from near Mono Lake in Mono County, California. This is at an elevation of from 6500 feet up and the season is therefore short. In the spring (May and June) almost all the butterflies are pale yellow, like *philodice*, but as the season progresses orange forms become more and more abundant until in the fall (August and September) only orange forms are found. I have just bred out here in Pasadena over a hundred individuals obtained from the egg (three orange females laid them) and a good percentage are yellow. Yet the population from whence they came was at the time 100% orange (I counted 69 orange and 2 white).

He adds this handwritten PS:

The population above was obviously heterozygous for yellow (or rather, no orange) but what besides rather stringent seasonal selection could account for the lack of yellow at this time of year. All individuals bred were under identical environmental conditions.

Again, Gerould's reply is missing, but he wrote in longhand on Hovanitz's letter that he sent four female *philodice* to him Special Delivery (88c postage noted!—Gerould meticulously recorded such expenses and expected compensation) on Sept.23 and they had arrived OK and began laying. Gerould seems to have started keeping carbons of his letters only in early October.

Hovanitz's next letter is dated Sept.17, 1940. He thanks Gerould for his comments on his published paper about the Satyrids *Oeneis chrysus* Doubleday and *Oe. ivalda* Mead and discusses his hypotheses about the adaptive value of their coloration. A week later he sent a long letter, entirely in longhand. The first half is about parallel color variation in *Melitaeini*, but then it switches abruptly to *Colias* and one must regret the absence of Gerould's letter:

Your comments on the changing *Colias* population in the east is (sic) interesting. I do not think, though, that the population will change very much unless the climate and vegetative cover also changes more. I believe it is the removal of the forest cover over the east which has allowed eurytheme to encroach upon that territory. Over the dry hills of California, the form *amphidusa* never used to occur but it is now the commonest form because of irrigated alfalfa and clover fields. I am certain that environmental conditions had nothing to do with the color variation of the brood I spoke of....I am now mating the adults at 25C and breeding them all through the life cycle at 25C with a ten hour light day.

Humidity is kept at 80 though this is rather difficult in this place where it is normally closer to 0...Later, I will send you counts of the variations in the brood though this will be difficult because of the variation in the orange. The greenness of the underside varies as well.

The yellow butterflies from the brood above have difficulty in mating. As yet, I have gotten no fertile eggs from them....

I am of the opinion that *eriphyle* is nothing but the normal spring brood of the populations living between the Sierra Nevada and the Mississippi Valley.

In eastern Washington this is the case, in Modoc and Lassen counties, California this is the case. In the area from Mono Lake

to the Owens Valley in Mono co., Calif. this is the case. All-year orange material is gotten only on the warm-winter western side... from B.C. to Mexico and east through Arizona and New Mexico so far as I know. Actually I do not consider them species hybrids but merely as individuals having, available or mixed in the population, genes for all the characters. Sterility seems to be a character not related to the color variation and present in distantly situated populations, but which is lost by sufficient inbreeding. Such sterile races seem to have gotten started and actually formed new species - for example, *Colias hartfordi* (*sic*) of California.... In geographically "isolated" strains where the distribution is continuous, I can only guess that an ecologic selection must keep the strains from completely mixing.

It is quite clear that Hovanitz is having trouble separating seasonal variation (polyphenism) from direct genetic color differences, and he is waffling over the same issues that troubled 19th-Century writers on *Colias*. He is ahead of his time in controlling the rearing environment strictly. The role of photoperiod in butterfly polyphenisms was completely unknown at this time; perhaps he got the idea it was relevant by encountering the then-current work on insect photoperiodism by Danilevskii in the Soviet Union.

He wrote again on Sept. 26 to acknowledge receipt of the live *philodice*, which had taken only two days to cross the country. But then he writes three pages in longhand giving precise data on the reared Mono County brood, including wing color and size by sex and number of days egg to adult. Then:

My own opinion on the population here (which will probably go for all Great Basin ones above 5000' elevation in the south and sea level in the North) is that it is a continually fluctuating condition with regard to the genes which determine orange coloration. Judging from the geographical distribution of the Pieridae and the seasonal variations, I would expect that the individuals showing an increase in orange would be selected against over winter and in those areas which have colder winters. Thus there would be the factor of environmental conditions acting both upon the phenotype directly and also upon the hereditary constituents. The individuals manifesting orange certainly cannot pass the winter safely at Mono Lake because there is no orange there in the spring brood.... With the increase in warm weather and warmer nights, the phenotypic and genotypic individuals would become more abundant....

The spring form from the coast of California has less orange than the summer form but never are all-yellow individuals obtained...

One is tempted to infer that his thinking here was colored by the just-published work of Timofeeff-Ressovsky (1940) on "cyclic polymorphism" in the ladybird beetle *Adalia*. As a graduate student in the Morgan lab he would definitely have known about it, whether or not he could read it in the original German.

Gerould replied in two parts, written on October 6 and 7. The first part concerns the invasion of the Northeast by *erytheme*; he discounts "removal of forest cover" as a cause, says the species is non-migratory,

and believes it has been accidentally introduced in commerce. In the second part he addresses the problem of *eriphyle*:

What you say about *eriphyle* interests me very much, for my experience with western yellow stocks east of the Rockies has not brought to light any seasonal change into orange. These stocks have been essentially true-breeding yellows, with a decided dash of orange on the under side of the forewings, distinguishing them from *philodice*. They mate readily with *philodice*, and I regard them and *philodice* as minor species sprung from the widely distributed *erytheme* and segregated from it by partial sterility, like *harfordi*.

I have raised large broods of yellow Colorado, Alberta, Nebraska and Kansas stocks, all similar in some respects but different from one another particularly in the tone of the under side, which as you know is strongly subject to seasonal influences.

In the discussion that follows it is evident that Gerould has had Wyoming material of the *C. alexandra* W.H. Edwards complex confounded with *eriphyle*. This was presumably the fault of his supplier, but in any case it confuses the results. He is left uncertain of what *eriphyle* really is, biologically, and closes:

Your letters have been very stimulating and useful to me and I am very grateful to you for them.

Hovanitz wrote back at once (Oct. 26). He noted that the Mono Lake population breeds on clovers, unlike pure *erytheme* that "just swarms" over alfalfa; "automobiles on the highway become plastered with them." Much of the following discussion reflects uncertainty on the part of both men as to the true nature of *eriphyle*, e.g.:

I prefer to consider each population as a unit and not try to make any species differentiations in this group. I look as (*sic*) this group as a whole and then look at each part of it as an adaptive complex...

He then speculates on the adaptive value of wing pigmentation, drawing parallels to *Melitaeines* and *Oeneis*, and causing Gerould to draw large question marks in the margins next to his most speculative comments. After explaining the fundamentals of the Mediterranean climate of cismontane California (and getting parts of it wrong), Hovanitz attacks the question of why only oranges occur west of the Sierra but the situation to the east is so confusing:

East of this divide, winters are cold north of about latitude 37 and the populations are mixed with a yellow form that is abundant in the spring.

In Lassen County in July—late spring for there—the population was about 50% yellow and 50% orange. [Note: *Colias* fly in April in Lassen County, and Hovanitz was sampling the second generation.—AMS]

Yellows were in copulation with oranges. The same was true for

Mono Lake in July. In late August as I wrote you the population was 100% orange phenotypically. Last weekend about 70 butterflies were captured and all were orange. This is the last for the season there as snow has fallen and the clover is dead.

...In the case of *eriphyle* I cannot consider the populations as specifically different from either *philodice* or *erytheme*. They seem to be rather perfect intermediate blocks connecting the two, with the adaptive gene complex different from either.

I am very grateful to you for your letters as one learns a great deal by discussing the problems. I am going to make periodic collections of material from Mono Lake next season (if Hitler doesn't come over!) and see if the seasonal selection of genes for orange- and yellow-colored forms has any truth in it.

Showing increasing impatience with Hovanitz's arm-waving, Gerould drew a big question mark at the phrase "seasonal selection." One wonders if he knew about Timofeeff-Ressovsky's work!

W.D. Field published his *Manual of the Butterflies of Kansas* in 1938, but apparently Hovanitz didn't read it until late in 1940. On Oct. 29 he wrote Gerould that Field treats *philodice* and *erytheme* as "subspecies" with *eriphyle* as a "form." But

This, however, doesn't mean anything. What is interesting is the fact that *eriphyle* here seems to be two-brooded and does not interbreed enough with *erytheme* to lose its identity. What more could be wanted for specific differentiation? I wonder if we don't have something here like the race A and B of *Drosophila pseudoobscura* at least in part [Note: these proved to be reproductively-isolated sibling species.—AMS]. Certainly, I believe the evidence of intergradation between some populations of the yellow form and the orange form is strong but in other cases, good physiological isolation seems to have developed.

On Nov. 18 Hovanitz wrote that he would be on the East coast for a meeting and could be reached care of Theodosius Dobzhansky at Columbia University. Oh, to have heard their conversations!

Gerould replied on Nov. 25 to Hovanitz's previous three communications. He offered to put up Hovanitz and his wife if they wanted to come for a visit. There is nothing to document whether this visit took place, as no more letters appear in the file until 1941. Most of this short communication is a dismissal of Hovanitz's suggestion that melanic coloration in *Colias* was adaptive in cold climates. He saw no mechanism for such adaptivity and thought melanism was an inevitable byproduct of slower metabolism at low temperatures. The adaptive value of seasonal polyphenism in *Colias* thermoregulation would not be demonstrated for another two decades, proving Hovanitz prescient. Hovanitz replied testily on New Year's Day, 1941 to a Gerould letter of Dec. 22 that we do not have. The subject is still adaptive melanism, and he insists that the climatic correlations virtually demand an adaptive explanation: "I am sorry that we differ so greatly in this regard." He then goes on

to reject some idea advanced by Gerould regarding recognizing backcrosses among the intermediates, but does not explain why.

At this point their correspondence seems decidedly strained. The next few letters concern rearing conditions and their impact on growth rates, survival and fertility; there is no talk of the *eriphyle* problem. Hovanitz sent Gerould a box of specimens to review. Gerould's notes on them are extant, but not a letter about them. The specimens were returned.

On May 20, 1941 Hovanitz wrote Gerould about discovering a new white mutant of *erytheme*, perhaps homologous to either the "whitish" or "blonde" mutants later described by Remington in eastern *philodice*. Then he reports the first spring census at Mono Lake (mid-May) and exclaims:

These results have decidedly surprised me and I'm afraid have thrown overboard my idea of seasonal selection, at least as supposed last year. Also, the overwintering of the orange and most white females is the only way I can account for these summer bred individuals (worn, too) at this time of year. I guess I have lots of surprises coming! Note the lack of the spring form of the orange type at least as it is known along the coast and the scarcity of white females in the spring emergence...

Hovanitz had fallen into the trap of assuming a single season of sampling would tell the full story. A perusal of the 66-generation record in Jahner *et al.* (2012) demonstrates the folly of single-year generalization. It is clear also that, just as he misread the seasonality in Lassen County, Hovanitz began sampling at Mono Lake a little later than he should have in 1941. Moreover, he was still missing the obvious solution to his problem: there is a routine upslope migration and colonization by *erytheme* in late spring, a phenomenon fully documented at Sierra Valley by Jahner *et al.* The summer brood phenotypes of *erytheme* that he censused in May 1941 had originated somewhere east and downslope of Mono Lake. By early July Hovanitz was reporting a ratio of 287 orange to 1 yellow female! In his letter of 20 July he announces discovery of a new population of yellows at Round Valley near Bishop, Inyo County (4500'). And for the first time he complains that his stocks are being ravaged by disease (presumably the classic *Colias* nuclear polyhedrosis virus, or "wilt disease"). We have no record of whether Gerould, who must have experienced virus problems also, had any useful advice, but during 1942 Hovanitz concluded that high water content of the food was a predisposing factor. He told Gerould he had largely overcome the problem by lowering the humidity in the rearing chambers.

During much of 1941 Hovanitz was preoccupied

with seasonal variation in the frequency of white females. Gerould, on Nov.17, cautions that as far as he can determine, the white female form is completely genetic and not subject to direct environmental influences. On Nov.21 Hovanitz wrote that on the advice of Professor Sturtevant, he had begun doing sight counts of the color phases in the field and was reasonably convinced this would not introduce much error in the estimates of frequency. Gerould (December 1) begged to disagree,

I am very skeptical about "counts," especially where the population is large. No human being can be quite sure whether any particular female has been seen by him already....Why adopt a very unreliable method when a perfectly reliable one (killing and preserving) is available and almost as convenient?

After a detailed discussion of the 1941 field data, his tone becomes quite harsh:

I hope that you will pardon me for saying that I think it is a pity for you to publish these misleading data until you have checked them up next July. Entomologists would readily and thoughtlessly accept them as supporting their traditional belief that there is something seasonal (excess of whites in the fall) about the white female. This is pure bunk, pseudo-science, and I would hate to have to attack it in print.

This inspired a long letter from Hovanitz on Dec. 3rd.

I am very sorry that my efforts to illustrate the variable ratio of white:orange females at Mono Lake has (*sic*) met with such violent repercussions. I think it will be noted that I have given no reason for the observed changes, nor do I harbor any....I do think that I have definitely shown a statistical and real change beyond that expected by random sampling alone. I do not know why they occurred....my abstract in Genetics states as much.

He goes on to review his field methods in detail, including a test involving mark-recapture [a method still quite novel in 1941] and adds:

I agree with you that no one on earth can know if the butterflies I have counted have been counted once, twice, or many times. But that is not the point. Genetics is a science based on probabilities; I have shown by my marking experiments just what the probabilities are of capturing one once, twice, thrice etc. They follow the normal mathematical curve.

If you intend to attack the traditional idea of entomologists that whites are of excess in the fall, I should prefer not to be mentioned since I do not believe in traditional ideas that are not based on fact, and I should not care to be misquoted.

....In conclusion, I feel safe to say that we both agree that what ideas are "pure bunk," "pseudoscience" etc. can only be proven or disproven by experiment or analysis. I should dislike very much to have you attack an analyzed case which I uphold as not "pure bunk" and have gone to great pains and expense to show is not.

This storm seems to have blown over, perhaps fading in significance in the wake of Pearl Harbor Day. There are no letters in the file until one from

Gerould on Dec.22 concerning his treatment of *eriphyle* in a forthcoming paper (Gerould, 1943). In it he asks Hovanitz to fact-check his treatment of data derived from the latter's work, as well as the accuracy of quotes from their correspondence. He states that backcross phenotypes can be recognized, a prior point of contention. Hovanitz sent him 1941 specimens to examine in detail. In a letter of Jan. 12, 1942 he reports on his conclusions from that study and reaffirms his belief that backcrosses can be recognized, asking Hovanitz why he believes to the contrary. He also tells Hovanitz that he keeps carbon copies of typewritten letters and there is no need to return the originals. [If Hovanitz had been doing that, it makes the gaps in the file that much more mysterious.] Gerould says that the proportion of intermediate phenotypes is quite high in some Northeastern populations, prompting Hovanitz to say in his reply of Jan.26 that he had met with Austin Clark in the winter of 1940 and "it will be difficult to convince him of the interrelations between *erytheme* and *eriphyle*." Clark (1932) had carefully documented the establishment of *erytheme* in the vicinity of the District of Columbia after 1929 and was convinced that it was driving *philodice* to extinction there through hybridization; he called it the "persecution of one butterfly by another." But it is unclear why Hovanitz thought he would be resistant to his (Hovanitz's) story.

On Feb.8, 1942 Hovanitz wrote that the proportion of phenotypic *erytheme-eriphyle* intermediates in Carson Valley, Nevada (Gardnerville, Minden, Carson City) is higher than of parents! He does not say how many samples, taken over what period, might be at issue.

Most of a Feb. 26 Hovanitz letter is devoted to attempts to parse the transmission genetics of ground color. Results seemed to differ depending on the source of the parents, and the matter remained unresolved. This makes the issue of recognizing backcrosses moot, if one has no estimate of the number of loci involved and whether or not they are simply additive. Both men were fully competent geneticists. The issue has not been definitively resolved today, although for our group a model of two loci with no dominance and simple additivity seems to fit the lab brood distributions well. At the end he declares:

Personally I do not think that there is specific differentiation (in a taxonomic sense) between *philodice*, *eriphyle* and *erytheme*, though they are quite different in their physiological and genetic behavior. I do think that *eriphyle* has much more in common with *philodice* than with *erytheme* (even besides color).

Gerould wrote "Correct" next to the last sentence. In his reply on March 10 he called that a "good letter," declaring that

When eriphyle is understood, then the hybridization problem can be approached with hope of success. Jumbling eriphyle with eurytheme, as one yellow-orange polymorphic species, would seem to me to be a concession to ignorance.

That paragraph could have been written before the entire correspondence had started! Had any meaningful progress been made toward clarifying the status of these entities?

THE LATER CAL TECH PERIOD

March 20, 1942; Hovanitz felt he had turned a corner.

Our second term is now just finished, as is likewise the grind of getting over a few of the requirements for the degree. I feel a little relief over being past that and having ahead mainly the work with *Colias*.

All the past year I have felt chained down and unable to do what I wanted at the time it was most desirable. Now if the war does not interfere I shall be able to accomplish something (I hope).

Gerould had previously asked about white female *eriphyle*, and Hovanitz said he was not certain of their existence. Gerould replied on April 9 that his assumption that they did was based on treating yellows from Kansas as *eriphyle*—but now he was having second thoughts on the matter; perhaps they were really *philodice*, or intermediate. He wrote to Field about this on May 12, but Field's reply is not in the file. In his 1943 paper, Gerould referred to his Kansas stocks as *philodice*, explicitly declaring (p. 424) that after initially treating them as *eriphyle*, he had changed his mind.

The debate over sight vs. removal counting was again joined in May. On May 8 Hovanitz reiterated his preference for sight counts. But then he dropped a bombshell:

I expect to make quite a comprehensive analysis of the whole *Colias* problem in North America...if the war does not interfere by removing me too soon. Data from all the major museums of the U.S. is either here or coming and I have data from a very many private collections everywhere. I also have much data from eyewitnesses as to the increase of eurytheme throughout the east. On top of this, the breeding data which I am now really beginning to get is coming along....I have not yet been able to figure out a way of putting this mating behavior on a statistical basis, since the *Drosophila* system obviously won't work.

Gerould's reply is missing, but Hovanitz wrote on May 13:

I don't know what to say about the two pages you have sent me. There are a very many points with which I disagree but as yet can put up little really good proof. Making much at this stage of the game is hazardous and I think subject to too much later alterations....

Perhaps it would be better to leave my criticisms go for

this paper unless you want them badly enough. My data and consequently my way of looking at these problems is changing all the time. There seems to be a decided "funny business" involved in the white female as well as the yellow and orange forms. I don't want to be pressed for an opinion at this stage of the game....I believe that I am just about to change my whole way of working at this problem and just when the light will dawn upon my at-present sleeping intellect, I don't know!

Again, Gerould's reply is missing. It is curious that Hovanitz retreated into declarations of uncertainty so quickly after announcing he was on the verge of a comprehensive synthesis!

On June 29 Hovanitz wrote to declare that he was now convinced [correctly] that the taxon *harfordi* (now spelled correctly) was a member of the *alexandra* group and irrelevant to the *eurytheme-eriphyle* story. On August 4, after a discussion of larval color and pattern in *Colias*, he declared:

I am discontinuing work on some of the stuff and soon will discontinue all but a mere line because of the war. I see little hope of staying out of the army beyond this fall or winter and there is no use being caught with too much on my hands. I wish that your paper were finished so that I could make a complete as possible job of *Colias* variation—geographical, genetical and environmental. The genetical part is going to be wholly inadequate at all events.

Gerould wrote in the margin that he had sent Hovanitz several sections of his own MS on Aug. 11. Hovanitz returned from a field trip a week later and wrote that he had not had time to read the material. But as for his own plans, he was still wavering:

Perhaps I should, however, correct or modify the impression that I gave in my last letter, namely, that I was giving everything up. On the contrary, I fully expect to get my material in shape and to turn it in as a thesis. I had in mind before only the discontinuance of the hybridization problem. My F1 and backcrosses have given my (sic) data which suggests in which direction I should work to carry out the analysis further. However this would entail too much work and preparation for the time possibly available. I have come to definite conclusions concerning the interrelations of eriphyle and eurytheme, and the status of the intermediates in the populations. I don't think they will be entirely the same as yours....I see no reason why I should not publish my data; I believe I have enough now to make a small monograph! (Including the complete geographical distribution and speciation in North America.)

There is not another letter from Gerould in the file until May 4, 1943. But there is a steady stream from Hovanitz.

On August 19, 1942 Hovanitz commented on Gerould's manuscript:

The conclusion that eriphyle is something genetically distinct from eurytheme, which you come to in your paper is the point that I have been trying to solve for these two years. Fortunately, we both come to the same conclusion. Unfortunately, I was not aware that you would or had come to the conclusion and hence one of the points upon which my thesis is based is a little exploded! I do

not know what your complete data is on eriphyle. The complete tale is far from being told even with my data but as I said I think that I had better stop the work now. I believe that my analysis of the two populations (Mono Lake and Round Valley) for 1941-42 and other populations elsewhere for white female frequency plus the genetic and physiologic data obtained should suffice [for the thesis]. Besides as I believe I have already stated I have made a complete study of the geographic distribution and variation of the forms throughout North and Central America and therefore can come to very definite conclusions as to the probable origins, migrations, ecology, hybridization of the forms. There are some genetic and physiologic questions related to the hybridization which I should have liked to have answered but the data of neither of us is complete enough for that....

It would be highly convenient if I might be able to have a copy of your manuscript when it is completed or whenever you can spare one, since I had intended to cover the literature on this subject. I note that you are covering a good deal of it in the pages you have sent me and it would save needless duplication to know how much you are covering.

There is no indication that Gerould sent any additional material in reply to this letter, but Hovanitz wrote again on September 1:

I do not think that your data is any more significant than eriphyle is a species than Edwards' was that it is not. Surely it breeds true but so do thousands of genetic mutants in *Drosophila* but certainly species are not made this way. I must say that from what I know at present of your data, that your conclusions do not have any stronger foundation than that of the taxonomist who knows the animal in the museum and field....

I have been working on the point from the geographical distribution, the genetic, the ecologic, physiologic, etc. points of view and have a tremendous lot of data to show its status. Still I have not made up my mind whether to call it a species or not. I rather think that I shall not.

I should abhor coming to the conclusion in print that I do not think your data are significant. Surely if I were in your place I would not come to a definite conclusion—to do so is identical to doing as a normal taxonomist does who "feels" what a species and subspecies is. I am sorry that I have to say this because I believe your work is important and represents a lot of effort. Taxonomists, however, are likely to view with skepticism the conclusions of geneticists...

And the very next day:

I hope that you will excuse my writing so much about whether I consider eriphyle a species or not... Recently I gave a talk in Berkeley and, pressed for a statement on this point, I said that you could flip a coin and take your pick that way. Of course it isn't as simple as that but I have just now decided that I find it more convenient and my paper much clearer if I don't come to any conclusion of that sort. Instead, I shall segregate my material in discussion to orange form and yellow form. Therefore I shall not object to your usage though I myself dislike calling them species and your usage of the term hybrid.

Apparently Gerould sent the complete text as it stood at that time, prompting a reply from Hovanitz on September 9:

You are truly kind to send me the copy of your manuscript even after the way I have written.—I am very sorry that I cannot

say that my opinions have changed since reading it, in fact they have become more definite.—This thing has placed me in a very bad spot and I don't know what to do. I can't possibly agree with you on your ideas of geographical distribution—they are based upon such skimpy data.

Yet if there is anyone I want more to agree with it is with you. To tell the truth which is not to be public information, I feel in about the same position as Sturtevant now is in with respect to Patterson on *Drosophila* species.

It is from data from everywhere that a ...general view of the problem [can be] obtained. ..Flops such as are being made daily on species problems or evolution treatises would not be made if persons would not be so narrow in their viewpoints....

I love arguments but I don't like the strained feelings that so often accompany them. As this is perhaps my last chance to write up a bit of work before going into war service, and perhaps the last of my work on butterflies, I think that it is fair to state my opinions on the problem. My general type of consideration will be clear from my last paper now in press in *The American Naturalist* on the "Geographical distribution and racial structure of *Argynnis callippe* in California and Nevada"....My method is to eliminate orthodoxy in geographical distribution work...

Don't misunderstand me. I think your paper excellent with the exception of the one point discussed so much...If I did not recognize its worth I would not mention it twice or even criticize it.

Hoping for the best,

Gerould's letter of Sept. 18, referred to in Hovanitz's of Sept. 25, is very unfortunately not present.

I am most grateful for your very long and excellent letter of Sept. 18, 1942.

Hovanitz revealed that he had received a six-month draft deferment to complete his work. He will concentrate on the white-female problem. The next day he wrote again with an urgent request, with just a hint of panic:

I trust that you will keep my work on these problems confidential and that you realize how important it is to me that this work remain original with me. Not having known how much you did on the yellow x orange crosses, I made unnecessary duplications. Many of the statements I make in my letters are based upon tedious research, likewise, the questions I ask, and it is very hard to see these things published before I have had the chance to present the evidence, at which time it has but second-hand value. You will, I trust, be considerate of this because I have been so willing to discuss with you all the time the results of my work as it has progressed.

On Jan. 29, 1943, Hovanitz wrote that all the formalities for his degree had been completed except filing the thesis; that he had accepted a commission as an entomologist in the Armed Forces; and that, preparatory to publishing his research, he had prepared a "short paper reorganizing the nomenclature of the group." Would Gerould review it? The MS is not in the file, but Gerould did comment on it. This MS, subsequently published (1943) in the *American Museum Novitates*, was apparently the first

place where Hovanitz, employing his announced view to global distributions, adopted a proposal by Austin Clark and combined the North American taxa *eurytheme*, *eriphyle* and *philodice* under the Palearctic taxon *chrysotheme* Esper, an action which when actually published was to be ignored by virtually all taxonomists—although it foreshadowed a wave of Holarctic lumping in butterfly nomenclature some thirty years later, which sunk a number of Nearctic taxa as subspecies of their (nomenclaturally older) Palearctic relatives. Hovanitz wrote a lengthy rejoinder to Gerould's (missing) critique, Feb. 5, 1943:

I am exceedingly grateful for your notes and comments on my paper. It is by the fair interexchange of ideas that science may profit. Nomenclature has two functions to perform—it must be convenient to use and should show as best as possible the relationships between things. I agree with you that *chrysotheme* is not the best name to use for the purpose in which I have used it. But neither *eurytheme* nor *philodice* is as satisfactory. Likewise I do not feel that a new name should be formed for this purpose, and therefore I have chosen the one least apt to form confusion. The chances are great that the morphological similarity between the Palearctic forms and the Nearctic forms will be justified in genetic similarity when breeding tests are made. If not, a change must be made. In how many living things have genetic tests been possible?...

Unfortunately, I do not feel that the nature of the case justifies the rash statements made with respect to my judgment...I have covered the literature and the museums of America. I have had genetic and ecologic experience with all these forms...a total of over 6000 museum specimens, a total of over 50,000 sampled individuals in California...I have bred the material as you have and I must say I appreciate the fact that you are the only other person beside myself who has done so. I will admit that Clark has not gone into great geographical detail but who has but myself on this group? I must assume that "snap judgments" as you put it must apply to all, then. Certainly the snap judgments of Edwards made years ago when little was known cannot be held forever....

Your geographical data and theories, with which I did not agree, are not the main part of your paper. Your genetic results are very accurate and good. They agree with mine and this is the main part....

I have an entirely different idea in the main point of difference between the orange and yellow races from what you apparently have. This is very briefly abstracted in the December number of the Bulletin of the Ecological Society of America 1942. Again, how can I make any comments on your work? There is nothing I find wrong with it but nonetheless I derive different conclusions on the basis of additional information. I admit and deeply acknowledge the fact that I could never have arrived at these conclusions if I had not had your earlier work....Surely it is not expected of scientists to maintain their original conclusions despite the advances in the science...It is seldom that I have found reason to question this faith I have had in you [to be objective].

Gerould wrote a rebuttal to this letter. For some reason he kept a rough draft of that rebuttal, although the finished letter is missing. From Gerould's draft, dated Feb. 8:

I had no intention of attributing a "snap judgment" to you, but rather to Mr. Clark whose proposed nomenclature you seemed to be adopting. If you must lump these American orange and

yellow species and subspecies together, as you seem bound to do as the result of your extensive studies, you need, as you say, one name. W.D. Field in '38 used *philodice* Godart. Field speaks of *C. philodice eurytheme*. Evidently you don't like that plan any better than I do, nor do you like *C. eurytheme*, coined later. If you must have trinomials, that is the name I should choose rather than the very little-studied European-Asiatic *chrysotheme*. Have you personally studied the morphology of *chrysotheme* in any way corresponding to the exhaustive studies you have devoted to the American forms?

He also took Hovanitz to task for using the word "data" as both singular and plural, and for errors in his use of Latin!

Hovanitz replied three days later, Feb. 11th.

Since we agree on the fundamentals of the *Colias* problem, does it not seem like a lot of unnecessary quibbling to argue about names? Names are only a means to an end. They are of use only so that we may know what we are talking about. If I used *philodice* as a name for all the N.A. forms or *eurytheme* for the same purpose, the restrictions which people have in their minds to each of these would be too difficult to overcome. *Chrysotheme* is admittedly not too logical but for the time being it is practical.

Colias lesbia of Argentina is nearly identical with *eurytheme* of North America. It has the same seasonal forms, the same habits, the same food-plant preferences. It is as much a pest on alfalfa in that region as *eurytheme* is in California. I would be tempted to classify *lesbia* with *eurytheme* and *chrysotheme*. ...It was to avoid unnecessary quibbling about names that I dropped out of taxonomy in the strict sense a long time ago.

On April 4 Hovanitz sent Gerould a genetics manuscript. Gerould's reply, as usual, is missing, but Hovanitz wrote a long response dated April 24. Most of the content is detailed and requires reference to the MS to be fully understood, but there is a trenchant comment on names again:

As far as the entomologists are concerned, I have not paid any attention to their nomenclatorial arguments of which there are many in the 19th Century. Personally, I think you have overrated the value of the statements of the 19th (sic) entomologists and have partially succumbed to their style of argument or "hunches."...Your play on names throughout [your] paper is an old taxonomists' trick which serves only to cover the true facts and relationships in this group.

The file contains a second Hovanitz letter also dated April 24.

Certainly, collectors in the field seem to know more about the true situation than anyone, whether a taxonomist in Ottawa, Ithaca, or Washington, or a geneticist in Hanover or Pasadena.

...I have found it necessary to ignore the statements which you have made. No field man is going to be able to reconcile his field knowledge with the statements you have made...May I ask, since I do not have your full paper, just what is the basis for your conclusion "that *eriphyle* of Pueblo, Colo is of an independent true-breeding minor species, as I have found it to be the case at other western localities?" ..."Breeding true" is a slim excuse for a "species." Since *eriphyle* is to you a minor species and *eurytheme* and *philodice* are major species, what is your definition of "major" and "minor" species?

Suddenly we have a carbon of Gerould's 5-page reply, dated May 4.

Instead of regarding our American "chrysosome" as one huge "species," as Clark and you do, I am more interested in a genetic approach to its evolution, in the integrity of its races and their physiological relations to one another. So long as your conception does not run counter to the facts as I have found them, I have no quarrel with it. ...So I still speak of the "eriphyle-philodice complex" as split off from *erytheme*....

How it [this group] got that way [so confusing] recently near Washington, D.C. should be evident to Austin Clark, though, disliking "hybridization," he revises the taxonomy and tries lumping. I had a good talk with him the last time I was in Washington. He knew everything about *Colias*. I admire his brilliance more than I trust his judgment and I like him personally.

Hovanitz wrote rather contritely on May 9 after reading this long epistle:

I have been thinking about the statements which you have made in your paper concerning the work of Scudder, Edwards etc. and believe that perhaps I have criticized [them] too harshly...

And even more contritely on May 14.

Your long letter of May 4 is appreciated and surely shows that the attitude I took in my previous letters was wholly unwarranted

But then Gerould's big 1943 paper came out. Hovanitz, August 13:

Although I like your paper very much I am sorry you went into points such as the distribution of the races, etc. that you knew I was going to cover thoroughly. I did not see these parts in your original manuscript. For this reason, I cannot help duplicating some of the portions in your paper, though I believe priority on my part was warranted here. You have been very fair in citing me in several places but I cannot help feel that many portions of your manuscript were written with my results in mind. I do feel that I have been fair with you in withholding publication of my data and in my citation of your data. You have certainly been kind in allowing me the prepublication use of the latter. It would have been nice had we been able to pool our data and make a really better work out of the whole, but I guess our differences are rather great...Monday, Aug. 16 I leave for Colombia [to work on mosquito genetics]...

Although the two men would remain in intermittent contact, the intense part of their relationship was over, and the enigmatic entity *eriphyle* would never be mentioned in their correspondence again.

AFTER CAL TECH

Hovanitz continued to write to Gerould from Colombia. His initial letters were strictly descriptive (Gerould had never been to South America) and did not refer to *Colias* at all. Gerould apparently took

the August 13 letter well, since Hovanitz opens his October 19 missive from Villavicencio thus:

Your attitude on my last letter from Pasadena is of such a nature that I cannot help but comment on it. Were more scientific people of your type I am sure the world would get along famously. You are certainly of the true scientific spirit...It is, I am finding, very difficult to find people with a fair and reasonable attitude such as yours.

On November 1 Gerould wrote to inquire if Hovanitz had found any *Colias* in Colombia. He mentioned that Austin Clark had failed to get him a series demonstrating the alleged absorption of *philodice* by *erytheme* in the D.C. area, and that there was a large "false brood" of *Colias* at Hanover, N.H. on November 1 that included both species and a putative hybrid. Hovanitz replied on December 7 that he was rearing the Andean *C. dimera* Doubleday on clover. Having returned to the States, he reestablished contact in a letter from Tallahassee, Fl, October 3, 1944, in it he mentions hearing from William T.M. Forbes to the effect that the oldest records of *erytheme* in the Northeast were under Palearctic species names (a common early mistake!) and had been missed, thereby creating a false impression of its absence there. On Nov. 11, 1944, writing from the Rockefeller Foundation in New York, he notes the occurrence of a "false brood" there and discusses the previously-commented-upon tendency of half-grown larvae of *philodice* to enter diapause while *erytheme* does not. After 1944 the letters become quite infrequent. By spring, 1945 Hovanitz is ensconced in Lee Dice's lab at the University of Michigan and tells Gerould he can hardly wait to see what *Colias* are up to. This initiates a new round of correspondence, with Hovanitz sending Gerould field data and observations, and Gerould commenting thereupon, all in a genial manner—except for occasional digs by Gerould at what he still sees as the folly of lumping everything into *chrysosome*. There is further discussion of whether or not there is a latitudinal and/or seasonal gradient in the frequency of white females. In 1947 Hovanitz went to the Arctic under the aegis of the Arctic Institute of North America to study *Colias* there and furnished Gerould a copy of his progress report, "Analysis of Natural Hybridization and Gene Frequencies in Arctic and Subarctic *Colias* butterflies." A second progress report was produced in 1948. Some of the publication from this work was delayed many years.

By 1950 Hovanitz was at the University of San Francisco and Gerould was working on his behalf to try to secure funding for *Colias* research, possibly in collaboration with Bjorn Petersen in Sweden, who had published recently in the journal *Evolution*. The last

item in the file is a handwritten note from Gerould to Hovanitz about this, dated January 30, 1950. Gerould died in 1961 at the age of 93. Hovanitz died in 1977 at the age of 62.

Coda

Reading the classic *Colias* papers of both men demonstrates inconsistencies in their points of view but hardly reveals the drama of their highly fraught relationship, especially during the latter half of Hovanitz's graduate studies when he became increasingly apprehensive about competition for priority. Having initiated the epistolary relationship with Gerould – initially not about *Colias* at all! – Hovanitz apparently came to believe he had told the older man too much of his thinking. At the same time, he could not restrain himself at times and would lash out intemperately at Gerould, only to backtrack and more or less apologize. Gerould, for his part, must have realized by some time in 1941 that Hovanitz was not just an acolyte, but a potential rival for "ownership" of the system. Having carried out breeding experiments over decades on an opportunistic basis, he clearly perceived a need to bring them together into one or more major publications – and soon. The perceived threat seems to affect his tone. He could be picky and a bit patronizing, and even brutal in his criticism ("bunk", "pseudoscience") but also seems to have accepted Hovanitz's apologies graciously. It is very unfortunate that some key letters are missing. How, for example, did Gerould defend his terms "minor species" and "major species" when called on them by Hovanitz? These terms were never adopted by any significant number of evolutionary biologists.

A noteworthy aspect of the correspondence is the lack of any discussion of the "reinforcement model" of speciation. The idea that selection against hybrids could lead to the deepening of prezygotic reproductive isolating mechanisms, and thus to the "completion" of speciation, had been entertained by Alfred Russel Wallace, but was explicitly advanced by Fisher (1930) in his *Genetical Theory of Natural Selection* and by Dobzhansky (1941) in *Genetics and the Origin of Species*. Both books would have been well-known to both men, and Dobzhansky's would have been read avidly in the genetics group at Cal Tech even as these discussions went forward. Reinforcement would seem to be potentially highly relevant to the conundrum of *eriphyle*, as well as the recent sympatry in the East. (Klots, in his field guide (1961), actually alluded to this in his discussion of *Colias*.) If *eurytheme* and *philodice/eriphyle* are indeed species, why do we not see selection at work to deepen prezygotic

reproductive isolating mechanisms between them? How could two species hybridize everywhere they came into contact and neither fuse together nor develop reproductive isolation, the two alternatives posited by neo-Darwinian theory? The discussions in these letters, like the papers, are highly taxon-specific and phenomenological and break no new theoretical ground; neither the Fisher nor the Dobzhansky book is cited in any of the papers from this period. The most theoretical treatment of *Colias* by Gerould was in a very early paper (1914) on mechanisms of speciation. Perhaps both men at this point subscribed to Muller's (1940, 1942) belief that reproductive isolating mechanisms arose incidentally to selection for physiological traits. This would not be surprising for Gerould; it would be much more surprising for Hovanitz. But Muller is not cited either. Nor did the extensive literature of apparently stable hybridization in plants come under scrutiny, despite the fact that with reference to hybridization, *Colias* act more like plants than animals normally do. The bibliographies of the *Colias* papers are remarkably parochial, given that the work was being performed at a time of intense and highly productive ferment in evolutionary biology and that both men had entrée to that ferment.

Both men were somewhat resistant to the ideas of the other, even when the matters that separated them in retrospect seem rather trivial. The only idea that seems to have disappeared entirely during their exchange was Hovanitz's of alternating seasonal selection, which was derived from a single season's observations at the very beginning of his career, and as noted may have been derivative from a current case of this sort in the literature. (The entire history examined here forcefully demonstrates the folly of hasty generalization from a handful of cases; Hovanitz was right in urging a broader view, though the geographic perspective he embraced was decidedly premature and seemingly "hyped" in his rhetoric.) Both men repeatedly circled the species question very warily. In this regard their genetic data should have given them an advantage over the 19th century entomologists disparaged by Hovanitz but taken seriously by Gerould. But they didn't, because there were no clear genetic criteria for deciding the question of speciation.

The factors maintaining the apparent equilibrium of hybridization in mixed *Colias* populations remain almost as murky as they were in 1950. The lack of introgression documented by Jahner *et al.* (2012) at Sierra Valley appears typical, but why? Why does larval diapause consistently fail to introgress into *eurytheme*, as noted by both Gerould and Hovanitz as well as by Jahner *et al.*? In retrospect it seems that these two

men took the system about as far as it could be taken in their time. It is remarkable that so few observations have been made of the situation in the field (none in eastern California between 1943 and 1981!) and that the pattern of spatial and seasonal occurrence of hybridization remains so very poorly documented. Many more such data are needed, but they will have to be combined with cutting-edge genomic analysis if we are ever to crack the enigma of *Colias*.

ACKNOWLEDGEMENTS

Sincere thanks go to Karen Hovanitz and to Andrea Bartelstein and Peter Carini of the Launer Special Collections Library, Dartmouth College, for searching for (but, alas, not finding) missing material from the Gerould-Hovanitz correspondence. The entire collection discussed here is now at the Launer Library.

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On the taxonomic status of *Tirumala tumanana* Semper, 1886 (Lepidoptera: Nymphalidae, Danainae)

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Abstract. Based on new information gathered from Scanning Electron Microscopy of the male alar and abdominal androconial organs, together with wing pattern and male genitalia characters, the nominal species *Tirumala tumanana* Semper, 1886, is demonstrated to be a distinct and geographically isolated species of *Tirumala* from extreme southern Philippines. For more than a century this taxon has been placed as a subspecies of *T. choaspes* (Butler, 1866) or of *T. limniace* (Cramer, 1775). The androconial organ data demonstrate that *T. tumanana* belongs to the *limniace* species group, and is not closely related to *T. choaspes*.

Key words: Lepidoptera, Nymphalidae, Danainae, *Tirumala*, species status, androconial organs, body size, *Tirumala tumanana*, *T. limniace*, *T. choaspes*, Philippines.

INTRODUCTION

Semper (1886) described what he considered to be a new species of milkweed butterfly from Tumanao, southern Philippines, as *Tirumala tumanana*. At that period Semper was still working in the taxonomic tradition that gave specific status to taxa from adjacent regions and islands if they presented discrete, even if small differences in color pattern. Soon after, however, systematists such as Karl Jordan working with Walter Rothschild introduced the polytypic species concept and with it, the advent of trinominal nomenclature (Mallet, 2004). By the time 'Seitz' started to appear some 20–25 years later, most butterfly workers had embraced the subspecies approach. As a result, despite continuing discovery of new regional and island forms, in groups such as the Danainae the number of full species recognized began to fall (Ackery & Vane-Wright, 1984: text-fig. 1).

In his description of *Tirumala tumanana*, Semper stated that it was most similar in appearance to *T. choaspes* (Butler, 1866) from Sulawesi, but he also compared it structurally with *T. orientalis* (Semper, 1879) from the Philippines. Today *orientalis* is treated as a subspecies of the widespread and highly polytypic *T. hamata* (Macleay, 1827).

Despite the outstanding appearance of *T. tumanana* ("it forms a very distinctive race": Ackery & Vane-Wright, 1984: 44), given the dominance of the polytypic species concept in butterfly classification, it is not surprising that throughout most of the 20th century Semper's *tumanana* was treated as a subspecies—either of *T. choaspes*, as presaged by Semper (1886), dealt with by Fruhstorfer (1910), Talbot (1943) and others, and followed by Ackery & Vane-Wright (1984)—or of *T. limniace* (Cramer, 1775), as proposed with good arguments by Morishita (1981: 461) and followed by Treadaway (1995). Despite this, Morishita (1981), Ackery & Vane-Wright (1984) and Vane-Wright & de Jong (2003) all also expressed the view that *tumanana* probably deserved specific rank. Most recently, based on male genitalia and wing pattern characters, Treadaway & Schroeder (2012) have returned it to full species status. The purpose of the paper is to assess this proposal in the light of new evidence from the androconial organs, together with the genitalia and wing pattern characters.

Received: 26 October 2011

Accepted: 1 March 2012

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SYNONYMY AND TYPE MATERIAL

Tirumala tumanana Semper, 1886

Tirumala tumanana Semper, 1886: 15. PHILIPPINES: Lectotype male, Sarangani Is., Tumanao. Senckenberg Museum, Frankfurt a. M. [examined HGS & CGT], here designated (see below).

Danaida (Tirumala) choaspes "Lokalform"; Fruhstorfer, 1910: 205.

Danaida (Tirumala) choaspes in part; Hulstaert, 1931: 49.

Tirumala choaspes tumanana; Bryk, 1937: 107.

Danaus choaspes Butler, in part; Talbot, 1943: 137.

Tirumala limniace tumanana; Morishita, 1981: 460–462, pl. 94 (3 figs.: ♂ ♀), and 2008: 3; Treadaway, 1995: 62.

Tirumala choaspes tumanana; D'Abreu, 1982: 206; Ackery & Vane-Wright, 1984: 44, 130, 198, pl. VII fig. 55.

Tirumala tumanana [distinct species?]; Vane-Wright & de Jong, 2003: 218.

Tirumala tumanana; Treadaway & Schroeder, 2012: 29, 55.

Tirumala tumanana was described by Georg Semper from two male specimens. Both represent, without doubt, the same taxon. One of these syntypes, labelled Sarangani Is., Tumanao, 24. vi. 1882, forewing length (fwl hereafter) 43 mm, is hereby designated lectotype of the nominal taxon *Tirumala tumanana* Semper, 1886, and has been labelled accordingly. The second syntype, with identical data but also labelled Gen.-Praep. 477 I. Schroeder, with fwl 47 mm, has been labelled paralectotype. Both specimens are in the Lepidoptera collection of the Senckenberg Museum, Frankfurt am Main.

CHARACTERS

Alar organs (Fig. 1). Butterflies of the subtribes Amaurina and Danaina produce pheromone transfer particles (PTPs hereafter) in several different ways (Brower *et al.*, 2010). Those of *Tirumala* are outstanding, as in all species of the genus they are produced by fragmentation of cushion scales formed within the pouched hindwing alar organs (Boppré & Vane-Wright, 1989). Hashimoto & Yata (2007, 2008a) carried out a systematic survey of the genus, using SEM, and found that, among the Asian species, the PTPs of *T. gautama* (Moore, 1877), *choaspes*, *ishmoides* Moore, 1883, *septentrionis* (Butler, 1874), *hamata* and *euploeoemorpha* (Howarth, Kawazoe & Sibatani, 1976) are all roughly rounded in form. Although each species appears to differ slightly in shape, they can all be considered similar to each other. In contrast, those of *T. limniace* and its putative sister species, the African *T. petiverana* (Doubleday, 1847) are distinctly polyhedral, not rounded. KH has now produced SEMs of *T. tumanana*, and its PTPs are polyhedral, very similar to *limniace* but not to *choaspes* (Fig. 1; cf. Hashimoto & Yata, 2007: figs. 8–10). The PTPs of

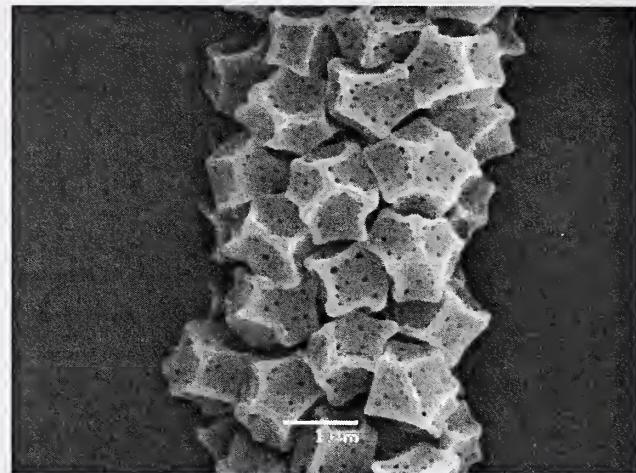


Figure 1. *Tirumala tumanana*. Short section of cushion scale within male alar organ, showing numerous, *limniace*-like polyhedral PTPs still attached (specimen in Osada Collection, Japan). Scale bar: 1 μ m. SEM by Kei Hashimoto.

T. choaspes are wrinkled, and in this respect similar to those of *T. gautama* (Hashimoto & Yata, 2008a: figs. 1a,b). The PTPs of the exclusively African clade represented by the polytypic species *T. formosa* (Godman, 1880) are very distinct (Boppré, 1976; Boppré & Fecher, 1977; Hashimoto & Yata, 2008a: fig. 2).

Abdominal hairpencils (Figs. 2, 3). The abdominal hairpencils of *Tirumala* are also unique among Amaurina and Danaina: they do not produce PTPs, and comprise only the one hair type, 'particle receiving hairs' (Boppré & Vane-Wright, 1989: 117). Hashimoto & Yata (in prep.) have now studied the microstructure of the receiving hairs in representatives of all currently recognised species of *Tirumala* except *T. alba* Chou & Gu, 1994 (see Discussion). Their results indicate that differences observable over the distal 40% of the length of the hairs can be used to group the species in the same way as the PTPs: (*petiverana* + *limniace*), (*gautama* + *choaspes*), (*septentrionis* + *ishmoides* + *hamata* + *euploeoemorpha*), with *formosa* again unique. *T. petiverana* and *T. limniace* have what can be described as 'dense granular processes', and this pattern is also seen in *T. tumanana* (Figs. 2, 3). In contrast, the surface ornamentation of the median-distal area of the hairs of *T. gautama* and *T. choaspes* has the crest processes very prominent, presenting a very different appearance. Species belonging to the *hamata* group exhibit ladder-like swirling patterns without dense granules, while *T. formosa* has very coarse grains (Hashimoto & Yata, unpubl. observations).

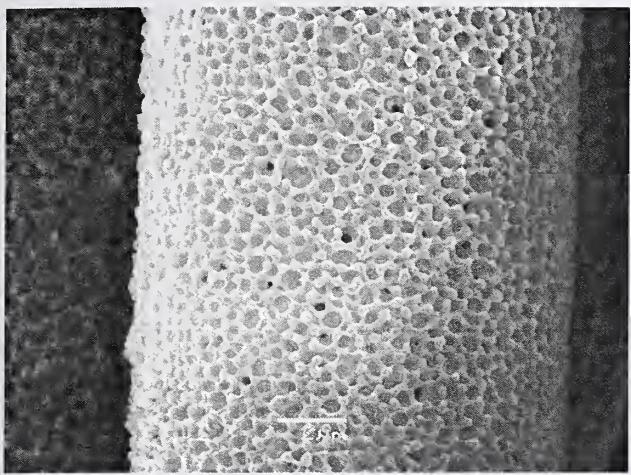


Figure 2. *Tirumala tumanana*. Short section of hair of male abdominal organ showing surface sculpture (specimen in Osada Collection, Japan). Scale bar: 2 μ m. SEM by Kei Hashimoto.

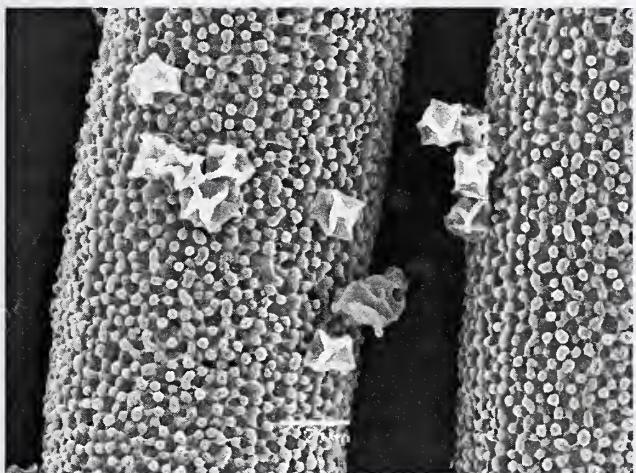


Figure 3. *Tirumala tumanana*. Short sections of abdominal organ hairs, with PTPs from the alar organ attached (specimen in Morishita Collection, Japan). Scale bar: 2 μ m. SEM by Kei Hashimoto.

Male genitalia (Figs. 4, 5). As demonstrated by Morishita (1981) and Ackery & Vane-Wright (1984), the highly asymmetrical phalli of *Tirumala* do offer characters that are useful for species separation. Phalli of all *Tirumala* species except *T. alba* (see Discussion) and *T. tumanana* have been illustrated by Hashimoto & Yata (2008b: 16). KH has recently had the opportunity to examine the genitalia of *tumanana*, and her drawings are presented as Fig. 4a, together with her earlier drawings of the phallus of *T. limniace* for comparison (Fig. 4b).

Working independently, HS and CGT have also examined the phalli of *T. tumanana* and *T. limniace*. HS notes the most obvious difference between them: in *limniace* the almost right-angled offset apex gives the effect of being inflated; the phallus it is also set with numerous microtrichia (Fig. 5b). In *tumanana* the phallus from the base to apex is equally wide in dorsal aspect, flatly curved subapically, and has a linear series of thorn-like processes (Fig. 5a).

Wing pattern (Figs. 6, 7). With respect to the wing pattern of *Tirumala tumanana*, Morishita (1981) noted that the “prominent subapical band composed of three bluish white spots [in forewing cells R_5 , M_1 , M_2] is a quite unique pattern otherwise not found in this genus” (Fig. 6). The three large spots in cells R_5 , M_1 , M_2 are submarginals, and far larger than the postdiscal spots located at the bases of these cells, these particular postdiscals in *tumanana* being almost obsolete. In contrast, *T. limniace* has the corresponding postdiscals well-marked, and usually far larger than the submarginals in the same cells (Fig. 7). Like *T. choaspes* but unlike *T. limniace*, *T. tumanana* lacks an outer postdiscal pale spot in forewing cell CuA_2 between the large basal pale marking and the small submarginal and marginal pale spots (Fig. 6). The underside melanic pattern of *T. tumanana* is very dark, almost black and almost as dark as the upperside, unlike most *T. limniace* in which the underside usually appears considerably paler than the upperside (Figs. 6, 7). Overall, the pattern of both sexes of *T. tumanana*, although so like other *Tirumala* in many respects, is instantly recognisable. In comparison to all subspecies of *T. limniace*, wing pattern alone could be considered justification for reinstating *T. tumanana* to species rank.

Adult size. ♂ forewing length 44.08 mm (mean of 17 specimens from South Cotabato: 15 from the Treadaway Collection, 2 from same source deposited in BMNH; observed range 36.8–47.0 mm; SD = 2.54 mm; 1 ♂ from Balut Island: 43 mm; 3 ♂ from Sarangani Island: 45 mm, 43 mm (lectotype), 45 mm (paralectotype).

♀ forewing length 45.38 mm (mean of 17 specimens from South Cotabato: 11 in Treadaway Collection, 6 from same source in BMNH; observed range 40.0–51.0 mm; SD = 2.78 mm; 1 ♀ from Balut Island: 42.5 mm.

With respect to the 17 males and 17 females from South Cotabato, although females have a mean forewing length 1.3 mm greater than the males, the difference based on these data is not statistically significant (two-sided *t*-test, unknown variances assumed equal: $t_{32df} = 1.423$, $p > 0.1$).

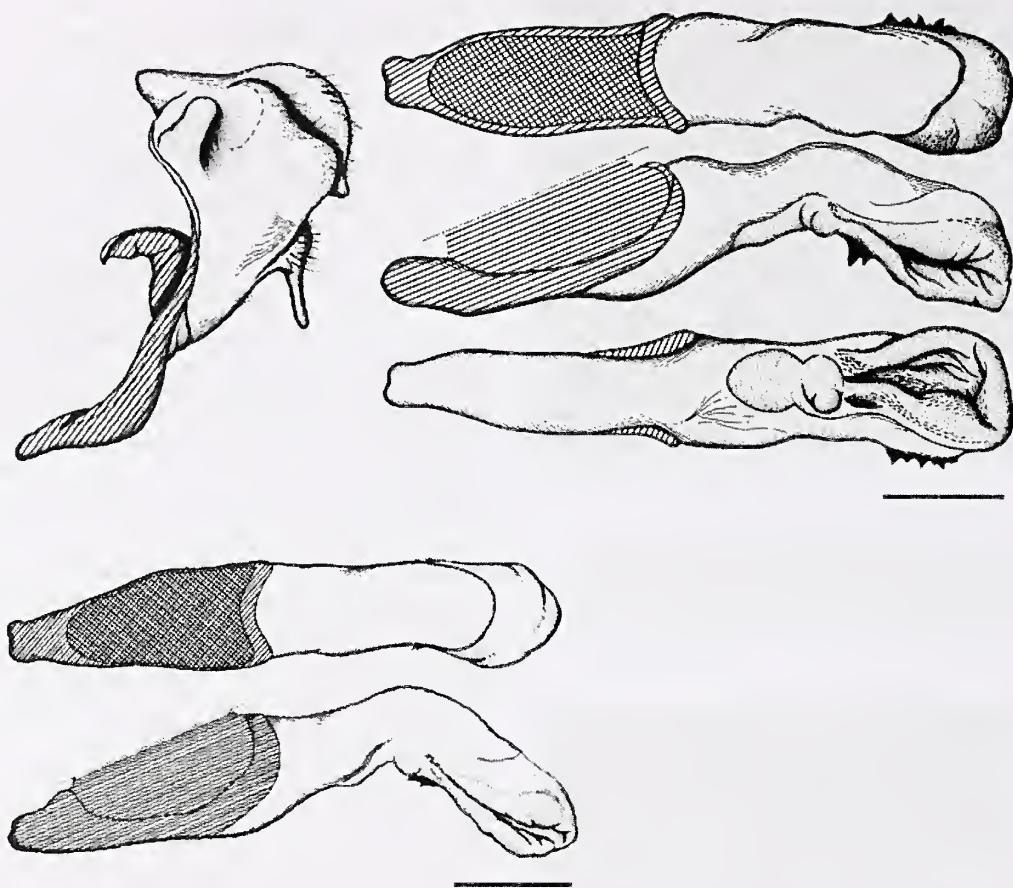


Figure 4. Male genitalia of *Tirumala*. 4a (above) — *T. tumanana* (Sarangani Island, 3.i.1980, Osada Collection); left: with phallus removed, lateral view; right: dorsal (upper), lateral (middle) and ventral (lower) views of the phallus (KH del.). 4b (below) — *T. limniace limniace*, phallus (Hainan); dorsal (upper) and lateral (lower) views (from Hashimoto & Yata, 2008b: fig. 11E). Scale bars (phallus): 1 mm.

DISTRIBUTION AND BIONOMICS

Distribution (Figs. 8, 9). *Tirumala tumanana* is only known to occur on the major island of Mindanao (Republic of the Philippines), and two small islands, Balut and Sarangani, situated immediately off its southernmost point (Figs. 8, 9). Old records for "Manila" (e.g. Adams and Rothschild collections, in BMNH) are certainly erroneous. Tumanao is the name of a small harbour on Sarangani, and the stream that flows into it. Until now all records for the main island were from the extreme south, in the provinces of South Cotabato and Davao del Sur. However, the discovery of two old specimens from Dapitan, in the north-eastern part of the island (Fig. 9), although these records must be questioned, raises the possibility that this species was once more widespread on Mindanao (see Discussion).

Known localities: Philippines, Mindanao.

Zamboanga Peninsula, Zamboanga de Norte: Dapitan (two males in National Museum of Wales labelled "Dapatan"). South Cotabato: T'boli (Lake Maughan, Mt. Parker; Siman; Mt. Busa; Mt. Matutum), and Lake Sebu. Davao del Sur: Sarangani Island (Tumanao), and Balut Island. Recorded at altitudes from 350–1900 m, during April–June and August–December.

Mindanao appears to be a genuine gap in the distribution of *T. limniace*, filled only in part by *T. tumanana* if this taxon is seen as its vicariant. *T. limniace* is also absent from Sumatra, eastern Borneo (Morishita, 1981), the Sangihe and Talaud archipelagos and northern Sulawesi (Vane-Wright & de Jong, 2003), and North and Central Maluku (Ackery & Vane-Wright, 1984). Moreover, it is very rare in the Malay Peninsula and its supposed presence in Sarawak has never been confirmed (Ackery & Vane-Wright, 1984). Thus, with respect to *limniace*, *T. tumanana* is completely isolated (Fig. 8). There



Figure 5. Phalluses of *Tirumala*. 5a (above) — *T. tumanana*. 5b (below) — *T. limniace*. Scale bar: 1 mm. (Light micrographs by Inge Schroeder, Senckenberg Museum.)

are no other records to confirm Dapitan, and it may be that this species, at least now, is entirely restricted to the Sarangani Islands and the mountainous areas of southern Mindanao just west of Sarangani Bay (Fig. 9).

Life history. The life history and larval foodplants of *Tirumala tumanana* are unknown. Fukuda & Lee (2009), who provided numerous excellent images of the early stages of *T. limniace*, found that on Taiwan *limniace* was monophagous on *Dregea volubilis* (Apocynaceae: Asclepiadoideae). In addition to *Dregea*, Ackery & Vane-Wright (1984: 199) recorded five other asclepiad genera as hosts: *Asclepias*, *Calotropis*, *Heterostemma*, *Hoya* and *Marsdenia*, together with *Crotalaria* (Fabaceae) and *Cocculus* (Menispermaceae), while Robinson *et al.* (2001) noted additional records for *Holarrhena* (Apocynaceae: Apocynoideae), *Tylophora* (Asclepiadoideae) and even *Saccharum* (Poaceae). Given the state of taxonomy of the Apocynaceae, and the problem of misidentifications of both butterflies and hostplants, most of these records must be viewed with caution (see discussion in Brower *et al.*, 2010). Fukuda & Lee (2009) noted that, in Japan, female *T. limniace* would not oviposit on *Cynanchum japonicum*, *Marsdenia tinctoria* var. *tomentosa*, *Heterostemma brownii*, *Tylophora tanakae* (Asclepiadoideae) or *Parsonsia* (Apocynoideae), while they would lay eggs on *Marsdenia tomentosa* and *Hoya carnosa*—but much preferred *Dregea volubilis*. Fukuda & Lee (2009) also give a list of 13 plants which, in Japan, *T. limniacea* larvae will not eat, including *Hoya carnosa*, *Marsdenia tinctoria* var. *tomentosa*, *Tylophora tanakae*, *T. ovata* and *Asclepias curassavica*. All non-dogbane family records are highly improbable

(Fukuda & Lee, 2009: 53). The most likely hostplants of *T. tumanana* will be found to belong to one or more species of Asclepiadoideae endemic to Mindanao.

DISCUSSION

During a brief visit to the National Museum of Wales, Cardiff, in November 2010, one of us (RIVW) came across two very old male specimens of *Tirumala tumanana* from the Rippon Collection, labelled “Dapatan” [= Dapitan, Mindanao]. The ultimate origin of this material is unknown, but Kirk-Spriggs (1995) lists H. Cuming (1791–1865), F.J.S. Parry (1810–1885) and C.G. Semper (1832–1893) as sources of Philippine material in Rippon’s collection. Dapitan, a medium-sized coastal town on the northern Zamboanga Peninsula, represents a significant extension of the known range of *T. tumanana* on Mindanao. While this must be questioned, at present there is no obvious reason to reject this historical record. Talbot (1943: 137) notes a specimen of *Tirumala ishmaoides* from Dapitan, so this is a known butterfly locality.

This discovery reminded RIVW of the status question affecting this taxon. He contacted Osamu Yata and KH in Japan, and CGT in Germany, to see if they were interested in addressing it. Subject to availability of material (subsequently obtained on loan), KH indicated she would be willing to undertake scanning electron micrography of the androconia, and dissections of male genitalia. CGT replied that, in collaboration with HGS, he was finalising a new catalogue of Philippine butterflies (Treadaway & Schroeder, 2012). In this work, based on wing pattern and male genitalia, they proposed to reinstate *T. tumanana* as a species close to but distinct from *T. limniace*, and not closely related to *T. choaspes*.

Our combined investigations fully endorse this last view. As described above, based on microstructure, the alar and abdominal androconial organs of *T. tumanana* are almost inseparable from those of *T. limniace*, with those of the latter being significantly different from *T. choaspes* and *T. hamata* (Hashimoto & Yata, 2007, 2008a, and unpublished observations). This then leaves only two possibilities: either *tumanana* is a subspecies of *limniace*, as Morishita (1981) proposed, or it is a species in its own right. As the two taxa are not known to co-occur, there is inevitably some degree of subjectivity in deciding on taxonomic rank.

Given the striking difference between the phalli of the two taxa (*tumanana* compared with several subspecies of *limniace*), and the unique and immediately recognizable wing pattern of both sexes of *tumanana*,



Figure 6. *Tirumala tumanana*, adult male, Sarangani Island, 3.i.1980 (Y. Osada) (Kitakyushu Museum). Left: upperside; right: underside. Forewing length: 50 mm. (Photographs: Kei Hashimoto.)



Figure 7. *Tirumala limniace limniace*, adult male, India, Khasi Hills (M. Nakayama Collection). Left: upperside; right: underside. Forewing length: 51 mm. (Photographs: Kei Hashimoto.)

we believe that the most appropriate status for *T. tumanana* is that of a distinct species in its own right, as proposed by Treadaway & Schroeder (2012).

T. tumanana therefore joins the small group of *Tirumala* species with restricted ranges—the others being *T. alba* known only from Hainan, and *T. euploeaomorpha* from the easternmost islands of the main Solomons archipelago (Tennent, 2002). The latter has been confirmed as a member of the *hamata* group by Hashimoto & Yata (2008a), and the possibility remains that *euploeaomorpha* is a vicariant, mimetic subspecies of *T. hamata* (see also discussion

in Tennent, 2002: 112). *T. alba* also requires further investigation, being the only currently recognised species of *Tirumala* yet to have its androconia studied in detail. Described from a unique specimen (Chou, 1994: 275, 755), it seems possible that *T. alba* is merely an albinotic aberration of *T. limniace limniace*, a species well-known from Hainan. Given these possibilities, the existence of *T. tumanana* as a distinct species narrowly endemic to the far southern Philippines is all the more remarkable.

Finally, a brief comment on adult size is called for. It is generally accepted that in most butterfly species

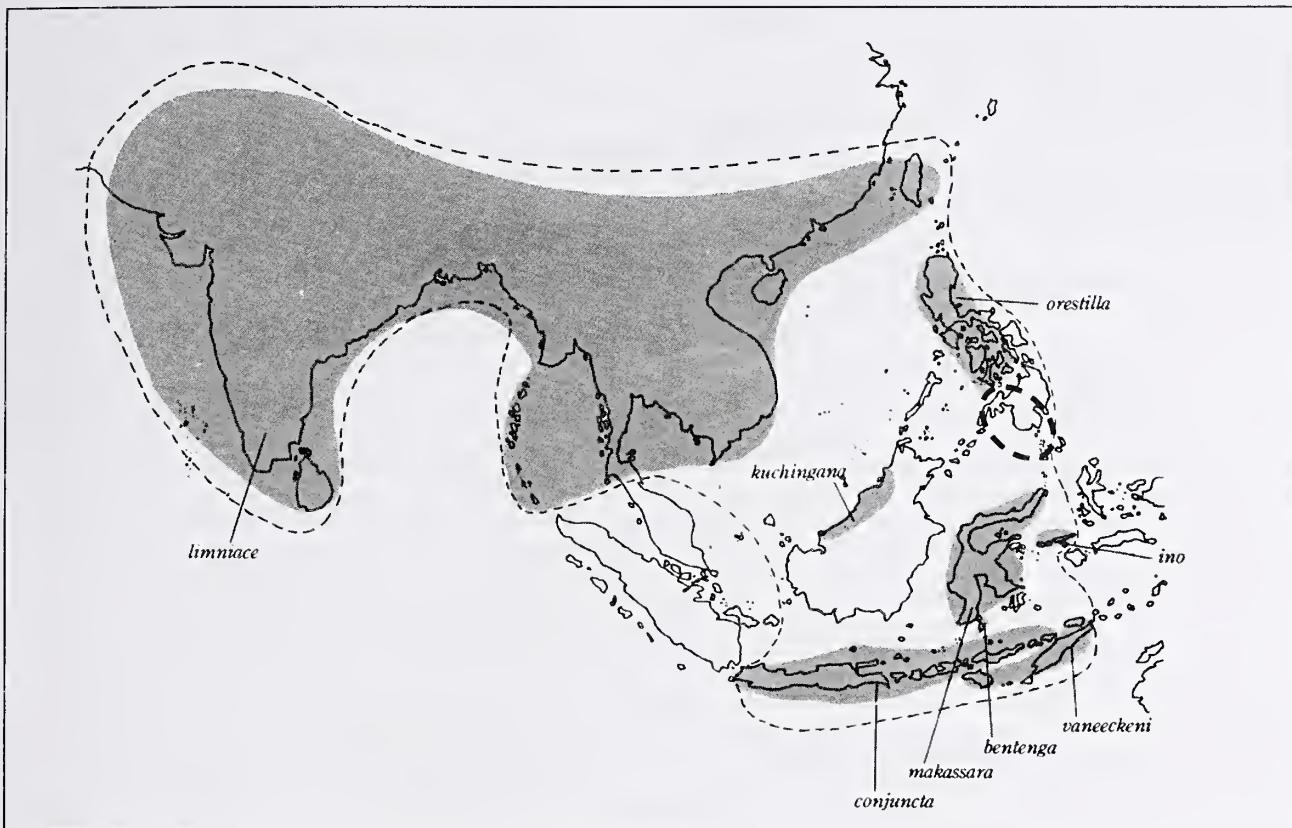


Figure 8. Distribution map of *Tirumala limniace*, which occurs as a series of subspecies from Afghanistan and Sri Lanka east to the northern and central Philippines, Sula Archipelago and Timor. Note the disjunctions, with 'gaps' in Borneo (where the presence of *T. l. kuchingana* (Moulton, 1915) has never been confirmed), Palawan, Sumatra, northern Sulawesi (not as shown here: see Vane-Wright & de Jong, 2003) and Mindanao. The species does occur rarely in the Malay Peninsula. Southern Mindanao is occupied by *T. tumanana* (pecked oval; for details see Fig. 9). Map modified from Morishita (1981: 460) with permission.

females are, on average, "larger" than males—as reflected by weight on eclosion (rarely measured) or forewing length (widely used as a 'standard' measure of butterfly size). There are exceptions, however, as recently documented for example by Liseki & Vane-Wright (2011) for two swallowtail species from Tanzania, in which the males undoubtedly have greater forewing mean lengths than their females.

This suggests the strong possibility that the sexes of some butterfly species may not differ in mean size, at least as measured by forewing length—or that if they do but the differences are small, this may only be detectable from large samples. Size is an important life-history trait that interacts with, for example, fecundity, longevity, and flight activity (Gilchrist, 1990). In Danainae it may be significant that males are particularly active in foraging for pyrrolizidine alkaloids (Brower *et al.*, 2010) and, when copulating pairs are disturbed, the male is the active partner in flight (Miller & Clench, 1968). To the best of our

knowledge sexual dimorphism in size has never been systematically investigated in the Danainae. The result obtained here that, on available data, male and female *Tirumala tumanana* are not significantly different in size, points to the need for systematic studies on size dimorphism in butterflies generally, and Danainae in particular.

ACKNOWLEDGEMENTS

KH wishes to thank Kyoichiro Ueda (Kitakyushu Museum of Natural History and Human History) for the loan of material collected by the late Yasunobu (Shilo) Osada, Kazuhiko Morishita for loan of material from his own collection, and Osamu Yata, Toyokazu Yoshida and Yasusuke Nishiyama for much assistance. RIVW wishes to acknowledge the kind help of Brian Levey (National Museum of Wales, Cardiff), and Clive Moncrieff for advice on statistics. The authors are grateful to Konrad Fiedler for



Figure 9. Map of Philippines, with well-established localities for *Tirumala tumanana* plotted as diamonds (those for the two small Sarangani Islands, Balut and Sarangani, offset). The single circular symbol indicates the general location of Dapitan, where the butterfly may also have occurred in the past (see text).

constructive criticism of the first draft. Finally, we are most grateful to Dr Morishita for kind permission to base Fig. 8 on his work.

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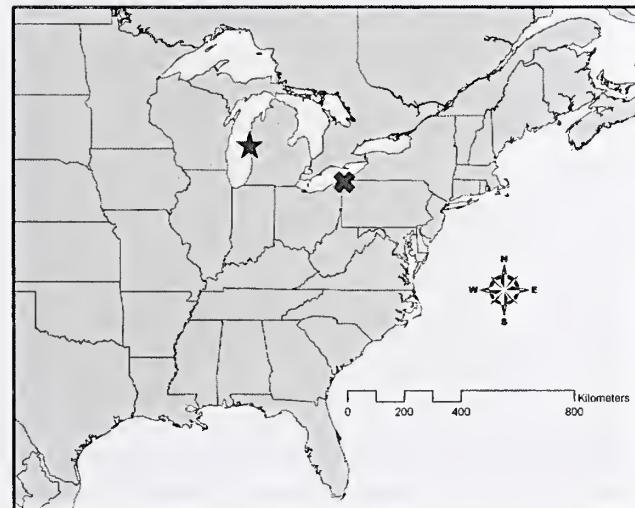
NOTE

Mortality of migrating monarch butterflies from a wind storm on the shore of Lake Michigan, USA

When monarch butterflies (*Danaus plexippus*) in eastern North America undertake their annual fall migration to wintering sites in central Mexico, they face numerous obstacles, and many do not survive the journey. Large water crossings in particular have long been known to be a source of mortality during migration; before the winter destination of eastern monarchs was known, Beal (1946) reported that he often found monarchs and other insects 'cast up' on the shore of Lake Erie. On one afternoon in September 1943, he collected 57 monarchs 'just above the water line' over 1.5 miles (2.41km) of beach (Beall, 1946). Other evidence that water crossings are risky comes from the monarch's reluctance to cross water during unfavorable winds (Schmidt-Koenig, 1985) and the fact that monarchs tagged along the Atlantic coastline have an extremely low recovery rate at the Mexican overwintering site (Garland & Davis, 2002; Brindza *et al.*, 2008; McCord & Davis, 2010). Large water bodies therefore appear to lead to substantial mortality of migrating monarchs. What is missing, however, from the collective evidence for the effect of water barriers, is first-hand accounts of mortality at such barriers. In this report, we summarize a series of observations submitted to the citizen-science program, Journey North (<http://www.learner.org/jnorth/>), regarding a mass mortality of migrating monarch butterflies at a location on the shore of Lake Michigan (Fig. 1) following an intense wind storm.

The storm in question was actually three back-to-back low-pressure systems that swept through the Midwest region of the US beginning on October 14, 2011. The national weather service for the Grand Rapids (MI) area described the systems as follows: The first low produced rain and cloud cover resulting in temperatures around normal from the 14th through

the 17th. The rain was light with multi-day totals mostly under an inch. Strong winds of 25 to 35mph (blowing westerly) were also felt across the area with winds gusting between 40 and 50 mph from late on the 14th into the 15th. The second low pressure system, which was more intense than the first, came a day later. Temperatures fell to below normal through the 22nd as heavy rain and strong (westerly) winds again lashed the area. Rainfall totals for the storm, mostly on the 19th and 20th, ranged from over an inch to nearly 3 inches across southwest lower Michigan. Winds gusted to between 40 and 50 mph with isolated sites experiencing gusts to near 60 mph. A third system came a day later; thunder and hail were reported at times from the 23rd through the 29th. This was accompanied by light to moderate rainfall. Temperatures were around or below normal through the end of October 2011 (National Climatic Data Center, 2012). The magnitude and duration of these storms can also be seen in a chart of the daily average and maximum wind speeds from the



Received: 3 March 2012

Accepted: 21 May 2012

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Figure 1. Location of the primary site of monarch butterfly mortality described in this article; a beach in Ludington, MI (star), on the eastern shore of Lake Michigan, USA. Letter X indicates the town of Erie, PA, on the eastern shore of Lake Erie, where additional weather data were obtained for comparison with Ludington.

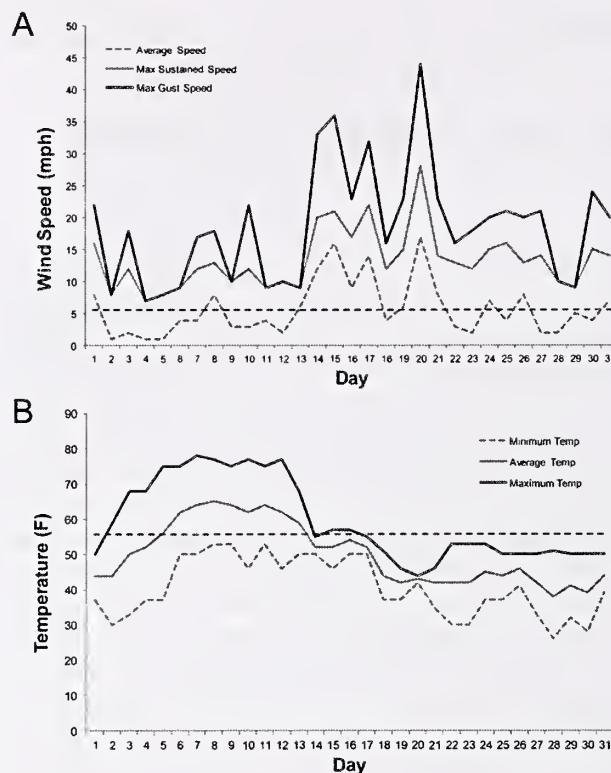


Figure 2. Chart showing daily wind speeds (A) and temperatures (B) for Ludington, MI (the location of the primary monarch report, see Fig. 1) in the month of October, 2011. Dotted line in A indicates the average wind speed for the month of October between the years 2000 and 2010 (6 mph). Dotted line in B indicates flight threshold temperature for monarch butterflies (55°F). Data obtained from Weather Underground (www.wunderground.com).

airport at Ludington, MI (Figure 2A; data courtesy of Weather Underground). In this chart, three distinct periods of exceptionally-high winds (i.e. higher than the 10-year average for this location) can be seen beginning on October 14th.

On October 15th, at the beginning of the storm, one observer (who used a pseudonym in her report and wishes to remain anonymous for this publication) visited a beach in the town of Ludington, MI (Figure 1), and witnessed the event. She subsequently reported to Journey North: “We discovered hundreds and hundreds of dead and dying monarchs on Stearns Beach (a city park beach in Ludington). They are being buried alive in the sand due to gale force winds. The wind was so strong that we could barely walk and yet here were hundreds, if not thousands, of monarchs clinging to anything they could find. We found them holding onto a seagull feather stuck in the sand, small pieces of dry grass, twigs, leaves, almost anything.”

On October 18th, this same observer visited

the Stearns Beach site again in order to conduct a more complete survey and to take photographs of the monarchs (Figs. 3A-F). On this day she walked approximately 200 meters of Stearns Beach and photographed every monarch she found – living or dead – that was either on the beach itself or up to 15 feet into the beach grass. In total she counted and photographed approximately 100 butterflies, though a few were not monarchs. Based on these images, one of us (Davis) estimated that 45% of the monarchs she saw were females. Also during this survey she noted that many of the dead monarchs were being eaten by various species of beetles (Fig. 3E).

With this anecdotal account it is difficult to estimate the actual number of monarchs killed (or damaged severely enough to incapacitate flight) by this storm, although it is likely in the thousands, if not hundreds of thousands. If we assume that there were 100 dead or dying monarchs per 200m of beach on Lake Michigan (based on the observer’s short survey), and there is 2,640 km of coastline on this lake (Wikipedia, 2012), by simple extrapolation we obtain 1,320,000 dead or dying monarchs. If even half of that number is closer to the real figure (660,000) the numbers are still exceptional. Moreover, this estimate is only for Lake Michigan, yet there are five great lakes and all are approximately within the central flyway of the monarch (Howard & Davis, 2009), and, this weather system appeared to encompass parts of Lake Huron, Lake Erie and Lake Ontario. It is also important to point out that Journey North received two additional reports of monarch mortality at other locations (from the same weather event) along the same coastline of Lake Michigan: one at Norton Shores and another at Muskegon, both on October 17. Neither of these was as detailed as the initial report, but they demonstrate that the effect of the storm was widespread on the coast of Lake Michigan.

While this particular storm appeared to be severe, we point out that such storm events are not uncommon during the fall throughout the migration flyways of the monarch. In fact, on further examination of weather data from the Ludington airport from 2001 through 2010 (from weather Underground, www.wunderground.com), we determined that on average, there were 13 days each fall (August through October) with maximum winds over 20 mph at this one location (Table 1). Plus there were nearly 12 days on average with gust speeds over 30 mph. Both of these wind speeds are similar to those from the wind storm of 2011 (Figure 2A), although not all of these days could be considered ‘storms’. A true wind storm would be where there were *consecutive* days with high winds, such

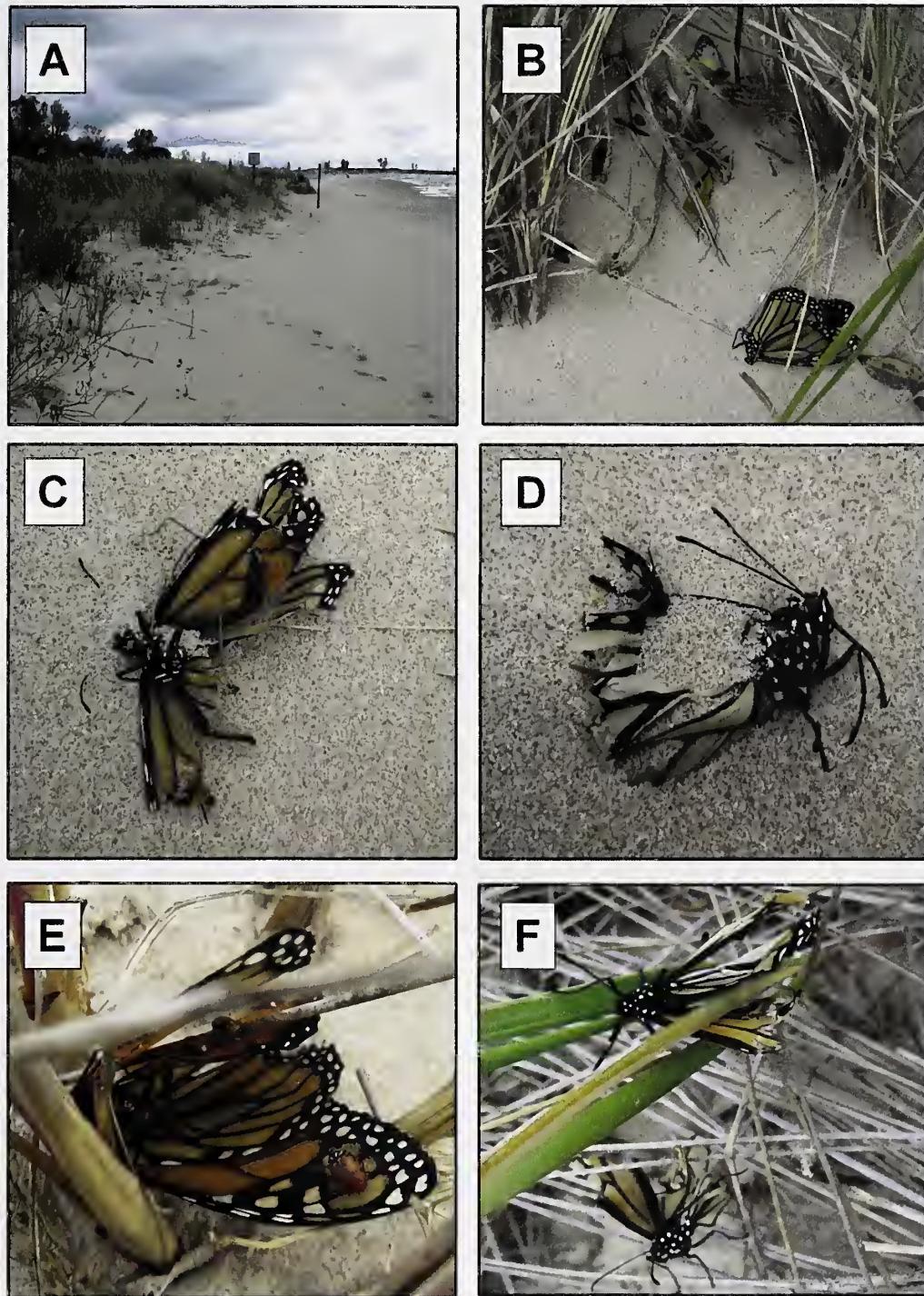


Figure 2. Photographs of (A) Stearns Beach (a city park in Ludington, MI) where monarch mortality was witnessed and (B-F) of monarch bodies, alive and dead, found along the beach. Note the beetles eating the dead monarch in E.

Table 1. Summary of the frequency of days with high winds in the months of August, September and October at Ludington, MI, (the Ludington airport weather station) from 2001 to 2010. Last column indicates the number of times there were 2 or more consecutive days with maximum winds above 20 mph (i.e. a prolonged storm similar to the one in question in this report). Weather data obtained from Weather Underground (www.wunderground.com).

Year	# days w/ max wind above 20 mph	# days w/ max gusts above 30 mph	# storms lasting 2+ consecutive days
2001	17	20	4
2002	14	12	3
2003	9	8	0
2004	14	15	3
2005	12	7	3
2006	9	10	2
2007	23	15	5
2008	8	4	0
2009	14	12	3
2010	11	14	4
Average	13.1	11.7	2.7

as occurred in 2011; at Ludington these occurred about 3 times each fall, on average, in the 10 years examined (Table 1). By comparison, examination of weather data for the same time frame (August–October only, from 2001–2010) at Erie, PA, a town on the eastern shore of Lake Erie (Figure 1) indicated that days with strong winds tend to occur even more frequently at this location each fall (Table 2). Clearly then, severe storms are not uncommon during the fall in the Great Lakes region, and it is very possible that storm-related mortality of migrating monarchs is also not uncommon. In fact, storms may not even be necessary to cause mortality over water crossings. Beall's (1946) early survey of dead monarchs on a beach on Lake Erie made no mention of a storm at all, and he indicated that the monarchs had likely drowned while attempting to cross the water. He further suggested that large water crossings may act as selection events for monarchs because the dead monarchs he collected appeared to be smaller on average than those captured elsewhere during the migration.

Beall's point about selection is well-taken. Whether it is from a major storm or simply from failure to cross large water bodies, a large proportion of monarchs likely die each year during migration. It is also likely that the ones that die are primarily the 'stragglers', or those that fell behind. This may have indeed been the case with the monarchs seen during the storm in October 2011; at a site on the north end of Lake Michigan (Peninsula Point) where migrating monarchs are counted each fall, over 90%

of all monarchs pass through before September 15 in most years (Meitner *et al.*, 2004). In addition, by mid-October the leading edge of the fall migration has usually reached 28°N latitude, or southern Texas (Howard & Davis, 2009). Therefore the monarchs seen on Lake Michigan (44°N latitude) during the storm were already approximately 1700km behind the rest of the migratory cohort, and about one month late. And finally, even if they were not killed by the storm, the temperatures in the region fell below the flight threshold for monarchs (13°C or 55°F; Masters *et al.*, 1988) following the storm (Figure 2B), so that all remaining butterflies in this area would ultimately have perished. All evidence therefore suggests that the monarchs witnessed by the observer on Lake Michigan were unlikely to ever complete the migration. Thus, the storm event of 2011 here could be considered an example natural selection acting to fine tune the monarch butterfly migration by weeding out 'suboptimal' individuals. And, if this happens every year, it is easy to see how natural selection would ensure that only the strongest and hardiest individuals would remain in the population.

From a scientific standpoint, it would be interesting in the future to compare certain characteristics of the 'straggler' monarchs to those that survive the journey, much in the way that Beall (1946) did, to find out what factors influence migratory success. While to our knowledge an investigation of this nature has never been done, a recent examination of tagging data did show how monarchs with wing damage and

Table 2. Summary of the frequency of days with high winds in the months of August, September and October at Erie, PA, from 2001 to 2010. Last column indicates the number of times there were 2 or more consecutive days with maximum winds above 20 mph (i.e. a prolonged storm similar to the one in question in this report). Weather data obtained from Weather Underground (www.wunderground.com).

Year	# days w/ max wind above 20 mph	# days w/ max gusts above 30 mph	# storms lasting 2+ consecutive days
2001	18	11	6
2002	8	6	1
2003	14	13	4
2004	13	8	5
2005	21	11	5
2006	34	20	7
2007	22	10	6
2008	22	15	3
2009	22	14	8
2010	28	22	8
Average	20.2	13	5.3

wear can fall behind because of their longer and more frequent stopovers (McCord & Davis, 2012). Moreover, infections with the protozoan parasite, *Ophryocystis elektroscirrha*, can also negatively impact flight ability (Bradley & Altizer, 2005), so that such individuals could fall behind as well. And consistent with Beall's (1946) results, Gibo & McCurdy (1993) showed how monarchs captured late in the migration (which could be considered stragglers) tended to have smaller forewing lengths than those captured during the peak of the migration. Other ideas that could be addressed in the future might be to examine wing shape of stragglers (Altizer & Davis, 2010) to see if it differs from typical migrants, or to compare the genomes of stragglers and successful migrants, which may provide insight into their innate migration programming.

Finally, we point out that identifying any source of annual mortality to monarch butterflies would be prudent, especially given the recent attention to tracking the long-term population status of eastern monarchs (Brower *et al.*, 2012; Davis, 2012). As such, we propose that future efforts should be aimed at surveying areas of coastline for dead monarchs on a regular basis in the fall, much in the way that McKenna *et al.* (2001) did along roadways in Illinois. This will allow for more precise estimates of annual mortality from storms or otherwise, which, if this report is any indication, may indeed be massive. In any case, this report certainly highlights the risks that all monarchs face when they undertake their long and arduous journey each fall.

ACKNOWLEDGEMENTS

We thank the many Journey North participants whose keen eyes continue to provide exceptional anecdotes and observations of monarch butterfly biology and behavior, and who help to advance scientific understanding of this amazing insect. We also thank Cindy Schmid, Journey North staff. Funding for Journey North is provided by Annenberg Learner. The manuscript was improved after helpful comments from two anonymous reviewers.

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Subspecies of the Violet Lacewing, *Cethosia myrina* (Nymphalidae: Heliconiinae), a protected butterfly from Sulawesi

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Abstract. Taxonomic problems concerning the diagnosis and nomenclature of the five subspecies of the protected Sulawesi endemic nymphalid butterfly *Cethosia myrina* are discussed. The correct name for the central and eastern Sulawesi subspecies is deemed to be *C. m. melancholica* Fruhstorfer, 1912, and that of the Buton and Muna subspecies *C. m. vanbemmeleni* Jurriaanse & Lindemans, 1918. *C. m. ribbei* Honrath, 1887, from the Banggai archipelago, is distinct. The nominate subspecies *C. m. myrina* Felder & Felder, 1867, from northern Sulawesi, is shown to be larger than *C. m. sarnada* Fruhstorfer, 1912, from southern Sulawesi, and the latter is reinstated from synonymy. A key to the subspecies and a discussion concerning their ontological status are presented.

Key words: nomenclature, synonymy, original spelling, infrasubspecific names, Sulawesi region

INTRODUCTION

"Our butterflies, sometimes the protected ones are ended up in the international market. An Indonesian protected, the lacewing butterfly (*Cethosia myrina*) can cost \$50 in an internet insect shop."

<http://wildlifewisdom.wordpress.com/2008/05/07/conservation-shock-one-earth-two-different-stories/>

The Indo-Australian genus *Cethosia* Fabricius, 1807, represents a very distinct clade of about 15 species, seen either as the only Old World representative of the otherwise exclusively neotropical Heliconiinae: Heliconiini (Penz & Peggie, 2003), or as the sister group of the pantropical Heliconiinae: Acraeini (Wahlberg *et al.*, 2009). Within *Cethosia*, *C. myrina* C. & R. Felder, 1867, is a very distinct species (Küppers, 2006; Müller & Beheregaray, 2010). *C. myrina* is endemic to the Sulawesi Region (*sensu* Vane-Wright & de Jong, 2003), occurring widely through much

of the main island of Sulawesi, and it is also known from the offshore islands of the Banggai Archipelago, and Buton and Muna (Fig. 1). According to Müller & Beheregaray (2010), *C. myrina* shares a sister-species relationship with *C. leschenault* Godart, 1823, from Timor.

The Violet Lacewing has the unusual distinction of being the only butterfly species other than the CITES-listed native birdwings (*Troides*, *Trogonoptera* and *Ornithoptera* species) currently protected under Indonesian law (Rhee *et al.*, 2004: Appendix 8; Peggie, 2011). Tsukada (1985: 296) recognised five subspecies: the nominate *Cethosia myrina myrina* from northern Sulawesi; the very similar *C. m. sarnada* Fruhstorfer, 1912, from southern Sulawesi; the striking *C. m. ribbei* Honrath, 1887, from Peleng (Banggai archipelago); a distinctive race from Buton island; and a race occurring in central to eastern Sulawesi. This arrangement was followed by Vane-Wright & de Jong (2003) in their synoptic account of the Sulawesi butterfly fauna. However, taxonomic problems have now emerged that potentially affect the delimitation and/or nomenclature of all *C. myrina* subspecies.

Once a taxon is subject to national and/or international law, the scientific community should do all it can to ensure that the names applied are as accurate and stable as possible. The purpose of this paper is to review the infraspecific taxonomy of *C. myrina* in the hope of resolving current uncertainties. The following five questions are addressed: what is

Received: 7 June 2011

Accepted: 29 February 2012

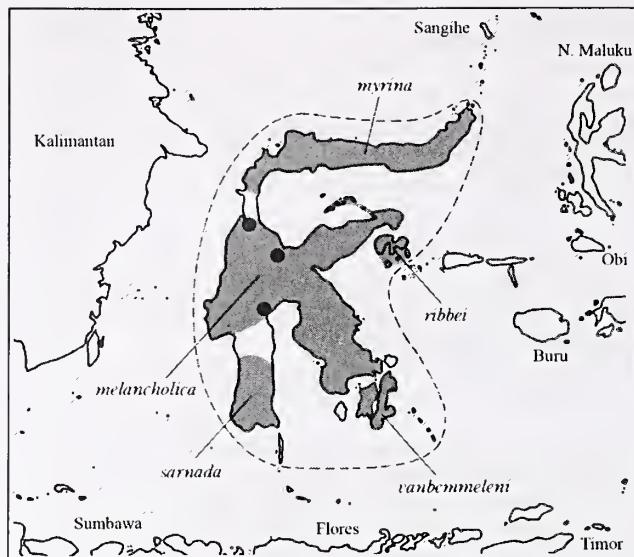


Figure 1. Distribution map of the five subspecies of *Cethosia myrina* recognised in this paper. *C. m. myrina*, *C. m. melancholica* and *C. m. sarnada* occur in separate areas of the main island of Sulawesi (Indonesia). *C. m. ribbei* is restricted to the Banggai archipelago, and *C. m. vanbemmeleni* to the south-eastern islands of Buton and Muna (Küppers, 2006), Muna being the smaller of the two, lying west of Buton. Black spots indicate three localities discussed in the text: Palu (most northerly), Mapane, and Palopo (most southerly). The distributions shown are 'envelopes'. Annotated map based on Tsukada (1985: 296).

the geographical range of the nominate subspecies; what is the taxonomic status of the southern Sulawesi population; what is the valid name for the central to eastern Sulawesi subspecies; what is the taxonomic status of the Banggai Archipelago population; and what is the valid name for the Buton and Muna subspecies?

Cethosia myrina myrina C. & R. Felder, 1867 (Figs. 2,3,6,7)

Cethosia aeole de Haan [MS]; C. & R. Felder, 1860: 103–104, pl. 1, fig. 2. “Celebes”. [Misidentification.]

Cethosia myrina C. & R. Felder, 1867: 386. Syntypes (*Van der Capellen, Lorquin, Wallace*). At least one syntype, northern Sulawesi, in BMNH [examined].

Cethosia myrina myrina C. & R. Felder; Fruhstorfer, 1912: 506; Tsukada, 1985: 296; Vane-Wright & de Jong, 2003: 237; Peggie, 2011: 16.

Cethosia myrina myrina C. & R. Felder; Küppers, 2006: 16, pl. 16, figs 5–8. [In part.]

Küppers (2006) departed from previous

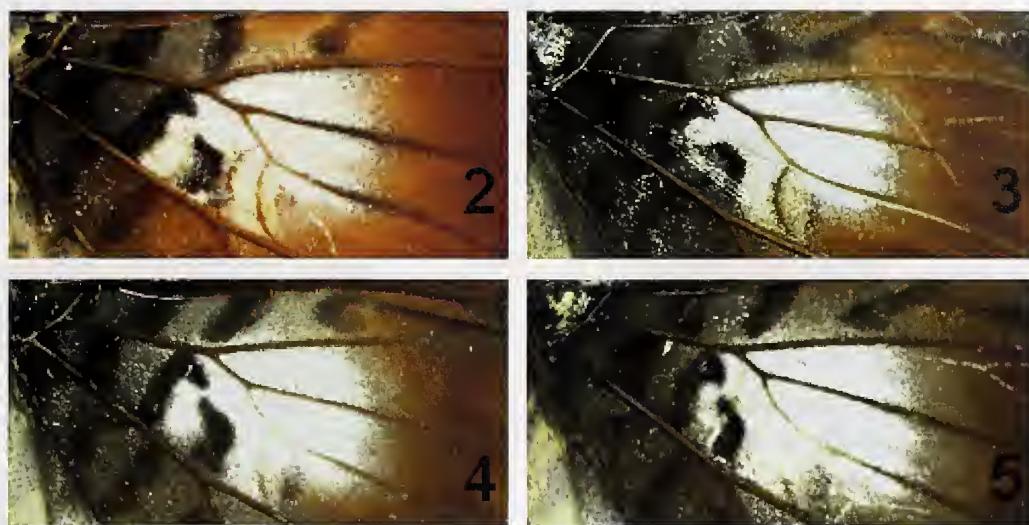
arrangements by including *C. m. sarnada* Fruhstorfer, 1912, described from the far south of Sulawesi, as a junior synonym of nominotypical *C. m. myrina*. Küppers gave the range of the revised nominate subspecies as northern, central and southern Sulawesi, with *C. m. melancholica* in central and eastern Sulawesi. Of the material he illustrates, a male from C-Sulawesi without further data appears somewhat intermediate between *melancholica* and typical *myrina* (Küppers, 2006: pl. 17, figs 1 & 2).

Based on colour pattern, *C. myrina* males from the northern peninsula of Sulawesi are indeed almost indistinguishable from males from the island's far south. However, in females the veins crossing the upperside hindwing white discal 'window' (notably cross-vein m_2 - m_3) are slightly more extensively marked by blackish scales in northern material compared with southern. This is evident in Figs 2–5, and can also be appreciated from the illustrations in Tsukada (1985: 77: figs 3,4,9,12)—although there is some variation.

Subjectively, the southern population also appears to show greater sexual dimorphism than the northern. In the north, females are generally bright orange, almost male-like (but with less iridescent magenta-violet coloration), whereas in the south-west females are mostly duller, brown rather than orange on the hindwings (although Küppers, 2006: pl. 16, fig. 8, illustrates a female from Papayoto, northern Sulawesi, which is similar to typical south-western females). Dimorphism is marked in the central *C. m. melancholica* and in *C. m. ribbei* from Banggai, in which the females are predominantly charcoal grey-brown, lacking, respectively, the striking iridescent violet or blue of their males. *C. m. vanbemmeleni* from Buton and Muna has the hindwing extensively orange in the male, with females duller in contrast (Küppers, 2006: pl. 18, figs 5–8).

Despite Küppers' (2006) account, given that *C. m. melancholica* definitely occurs at Palu (Figs 1 & 8), it seems that *C. m. myrina* populations to the north of Palu are not directly connected with the very similar looking populations found in the far southwest. If so, then *C. m. myrina* sensu Küppers (2006) is a polytopic subspecies (Mayr, 1963: 349), with two major geographical subdivisions separated by a distinct subspecies (*melancholica*). This would seem to imply that gene flow between these subdivisions would be more restricted than within them. Given that the colour pattern differences in support of this possibility are slight and not entirely convincing, what other characters systems are available?

A molecular investigation would likely be the most powerful tool to answer this question but, as yet, no relevant data are available. Although Müller



Figures 2–5. Upperside hindwing discal cell of four female *Cethosia myrina*. 2, *C. m. myrina*, "N. Celebes"; 3, *C. m. myrina*, "N. Celebes"; 4, *C. m. sarnada*, "Maros", southern Sulawesi; 5, *C. m. sarnada*, "Bonthain", southern Sulawesi (all specimens in BMNH, London).

& Beheregaray (2010) analysed DNA data for two specimens attributed to "*C. myrina myrina*", both came from southern Sulawesi. One was from "Salawatang, south Sulawesi" (Müller & Beheregaray, 2010), the other from Beso Valley, less than 100 km south of Palu (southern tip of Lore Lindu National Park, collected at 1450 m on 10.xi.2002, at 01°39.17'S, 120°10.64'E: Christian H. Schulze, pers. comm.). A specimen of *C. myrina ribbei* (Banggai archipelago) also analysed by Müller & Beheregaray showed very significant differentiation from these two (see below), indicating that a molecular study might well give a useful result. Available data on size, however, does give some evidence of north-south differentiation.

Males of *C. myrina* from northern Sulawesi (north of Palu) in the BMNH collection have the following forewing lengths (measured in mm): 49.2, 50.4, 52.7, 54.0, 49.1, 50.1, 55.4, 52.0, 53.2, 54.0, 57.2, 55.2, 54.0, 51.7, 52.3 ($N = 15$, mean ± 1 SD = 52.70 ± 2.36 mm). For females the values are: 52.6, 56.5, 51.3, 53.3, 53.8, 56.0, 58.8, 55.0, 57.1, 60.7, 57.3, 53.0 ($N = 12$, mean = 55.45 ± 2.78 mm).

Males from southern Sulawesi (well to the south of Palu) are recorded as: 42.0, 42.3, 44.4, 45.4, 45.2, 47.5, 46.0, 49.5, 49.5, 51.1, 51.1, 50.5, 51.5, 50.8, 54.0, 51.1, 47.8, 47.2, 43.8, 47.0, 48.9, 48.2, 52.2 ($N = 23$, mean = 48.13 ± 3.26 mm). Southern females have the following: 47.2, 50.0, 54.1, 53.0, 58.0, 58.3, 56.0, 57.7, 43.7, 47.0, 51.6, 53.7, 54.0, 50.9 ($N = 14$, mean = 52.51 ± 4.43 mm).

These data suggest that, despite considerable

overlap, the mean forewing length of male *myrina* in northern Sulawesi is almost 5 mm greater than in southern Sulawesi. If the mean forewing lengths are squared, this suggests northern males could on average have a wing area up to 20% greater in extent than their southern counterparts. But is this difference statistically significant?

Comparing the forewing lengths of the two male samples (two-tailed *t*-test with unknown variances not assumed to be equal) gives a *t*-value of 4.68 (calculated manually, and checked using online program at http://www.physics.csbsju.edu/stats/t-test_bulk_form.html). With 36 degrees of freedom, this equates to a probability of less than 0.0001 for the null hypothesis that the two population means are the same.

The northern females sampled have a mean forewing length 2.9 mm greater than the southern females, with a *t*-value of 1.52. With 24 degrees of freedom, the probability for the null hypothesis that the two population means are the same is 0.14. Thus the observed difference in mean size for the female samples is not significant. However, this difference does have the same sign as that of the males, which do show a significant difference. On present evidence, it can be concluded that *C. myrina* from northern Sulawesi are larger than those found in the southern part of the island.

The difference in size could be clinal, but there are not enough data of sufficient quality available to explore this possibility. Another explanation



Figures 6–11. Images of the five subspecies of *Cethosia myrina* (all 'halved', with ups left and uns right). **6, 7**, *C. m. myrina* C. & R. Felder: male, "N. Celebes" (6), female, "N. Celebes" (7); **8**, *C. m. melancholica* Fruhstorfer, male, "G. Tompoe, Paloe, 2700 ft., West Celebes, Jan. 1937, J.P.A. Kalis"; **9**, *C. m. ribbei* Honrath, male, "Bangkei, H. Kühn, 1885"; **10**, *C. m. sarnada* Fruhstorfer, male, "région basse entre Maros & Tjamba W. Doherty 1896"; **11**, *C. m. vanbemmeleni* Jurriaanse & Lindemans, male, "Matanauwe, c. 8 km E, Bau Bau-Lasalimu Rd., Pulau Buton, 250 m, 24.viii.2000, K. Willmott". All specimens in BMNH London except Fig. 11, courtesy of Dr Keith Willmott.

could be the temperature-size rule, or its ecological counterpart, Bergmann's rule (Ray, 1960; Kingsolver & Huey, 2008; Meiri, 2011). The first rule indicates that, in general, the lower the temperature at which the progeny of a single pairing are raised, the larger the adults will be. There are, however, some well-

documented exceptions, notably in grasshoppers (e.g. Walters & Hassall, 2006). Bergmann's rule, originally proposed for endotherms, can be defined as "a tendency of organisms to be smaller at high temperatures and low latitudes and larger at low temperatures and high latitudes" (Meiri, 2011: 205).

In ectotherms, however, there are many exceptions, with taxa showing so-called converse-Bergmann latitudinal variation (see discussion and references in Kingsolver & Huey, 2008). For tropical and south-temperate butterflies, at least, there is some empirical evidence that Bergmann's rule can apply (Baker, 1972; Vane-Wright *et al.*, 1975). However, Brehm & Fiedler (2004) did not find any such correlation in a study of altitudinal size variation based on a sample of almost 1000 species of geometrid moths from southern Ecuador. At high latitudes, converse-Bergmann patterns of latitudinal intraspecific variation are frequent in butterflies (Nylin & Svärd, 1991; Nygren *et al.*, 2008).

In the present case, the sample of northern Sulawesi *C. myrina* comes from a region that lies almost exactly between the equator and 2° N, while the southern sample comes from localities bounded by about 3–5.5° S. Thus, were the temperature-size or Bergmann's rules to apply to this species, other factors being equal (which they may not be) then, if anything, one would expect to find the reverse of the result reported above: the northern Sulawesi sample would be expected to be smaller than the southern. While there seems little point in discussing this further without data from far more extensive fieldwork and laboratory rearings, this finding gives some additional reason to consider that the size difference found between these two aggregates or populations, northern versus southern, could and perhaps should be treated as significant. On this basis, and their separation by the central Sulawesi race in the Palu region, it is proposed that the northern and southern groups should continue to be regarded as separate subspecies, *C. myrina myrina* and *C. m. sarnada*, until such time as they can be more extensively investigated using new data from molecular studies and extensive morphometrics.

***Cethosia myrina sarnada* Fruhstorfer, 1912, subsp. rev. (Figs 4,5,10)**

Cethosia myrina sarnada Fruhstorfer, 1912: 506, Pl. 110d (as "myrina"). Male, female syntypes, "Süd-Celebes, Bonthain" (= Mt Lompobattang) (H. Fruhstorfer).

Cethosia myrina sarnada Fruhstorfer; Tsukada, 1985: 296; Vane-Wright & de Jong, 2003: 237; Peggie, 2011: 16,17.

Cethosia myrina myrina C. & R. Felder; Küppers, 2006: 16, pl. 17, figs 3–4. [In part.]

Described from at least one male and one female (syntypes) from "Süd-Celebes, Bonthain" (= Mt.

Lompobattang), where it occurs, according to Fruhstorfer (1912: 506), up to 1000 m. The type material should be sought in the Muséum National d'Histoire Naturelle (MNHN), Paris. Note: the original description, even when interpreted by a native German-speaking lepidopterist (Michael Boppré, pers. comm.), is ambiguous regarding the type material. Only those specimens mentioned from "Bonthain" are certainly included. The type-locality is hereby restricted to the slopes of Mt. Lompobattang.

As discussed above, with some good reason Küppers (2006) departed from previous arrangements by placing *C. m. sarnada* as a junior synonym of *C. m. myrina*. Whether this does or does not create a polytopic subspecies, separated into two major halves by *C. m. melancholica* (Fig. 1), remains to be seen. On the evidence presented above, it is provisionally concluded that the traditional arrangement should be maintained, with *sarnada* applied to populations of *C. myrina* from the main south-western peninsula of Sulawesi. The only clear difference on present evidence is size, with male *sarnada* having a significantly shorter mean forewing length. The small difference noted in the coloration of the hindwing discal area of females (Figs 2–5) is not entirely convincing but might yet prove to be a good separation. Molecular and extensive biometric investigations are much to be desired.

***Cethosia myrina melancholica* Fruhstorfer, 1912 (Fig. 8)**

Cethosia myrina Felder, ♀ forma *melancholia* [sic] Fruhstorfer, 1909: 229. "Ost-Celebes, Mapane, Februar 1895, Drs. Sarasin leg., 1 ♀ Coll. Fruhstorfer, 2 ♀♀ Museum Basel, eine Anzahl ♀♀ von Paloppo, Coll. Martin." [Infrasubspecific.]

Cethosia myrina melancholica Fruhstorfer, 1912: 506, pl. 110d. INDONESIA: Sulawesi. Unspecified number of male and female syntypes: East Sulawesi, Palopo (*Martin*); and Gulf of Tomini, Mapane, north of Lake Poso (*Sarasin*). [Specimens probably in MNHN Paris; ZSBS Munich; and NM Basel.] [Not examined.]

Cethosia myrina [♀ form] *melancholica* Fruhstorfer: Martin, 1921: 139.

Cethosia myrina melancholica Fruhstorfer, 1909 [sic]: Tsukada, 1985: 77, 78, 296; Vane-Wright & de Jong, 2003: 237; Küppers, 2006: 16, pl. 17.

Cethosia myrina melancholica Fruhstorfer: Talbot, 1923: 41; D'Abraera, 1985: 273.

Cethosia myrina melancholica Fruhstorfer, 1912: Peggie, 2011: 16.

Central, eastern and south-eastern Sulawesi are

occupied by a bluish-violet subspecies (which includes a population on the Togian islands, Tomini Bay) that on the main island appears to separate the northern and southern races (Tsukada, 1985: 296; Fig. 1). This central race, distinguishable from the blue *C. m. ribbei* (see below), is currently referred to in major faunal works as *C. m. melancholica* Fruhstorfer, 1909 (e.g. Tsukada, 1985; D'Abra, 1985; Vane-Wright & de Jong, 2003). Unfortunately, it appears necessary to change either the spelling of this name or its date of publication. It is proposed here that it is better to change the date rather than the spelling.

The first name to be applied to *C. myrina* from this part of Sulawesi was introduced in the form “*Cethosia myrina* Felder, ♀ forma *melancholia* [sic]” (Fruhstorfer, 1909: 229). Fruhstorfer's original description refers only to the female sex and only lists female material. The name *melancholia* Fruhstorfer, 1909, was therefore conferred on an “infrasubspecific entity” in terms of the International Code of Zoological Nomenclature, as it refers to “specimen(s) within a species differing from other specimens in consequence of intrapopulational variability (e.g. opposite sexes . . . seasonal forms . . . variants of noninterrupted variability or polymorphism . . .)” (ICZN, 1999: 107).

However, under the current Code (ICZN, 1999: Articles 10.2, 45.6), the fact that this name was originally proposed as an infrasubspecific entity does not automatically bar it from use as an available name of the species group “if, before 1985, it was either adopted as the valid name of a species or subspecies or was treated as a senior homonym.” (ICZN, 1999: Art. 45.6.4.1).

Fruhstorfer's (1912) account of *C. myrina* in ‘Seitz’ recognised four subspecies, and this was the system, with addition of the island race from Buton, adopted by Tsukada (1985). For whatever reason, in recognising the existence of a central to eastern Sulawesi race, Fruhstorfer (1912) used a different spelling from his 1909 form name. Thus the two names could be seen as independent proposals. Moreover, they cannot be construed as homonyms—and it is the later, 1912 spelling that has been adopted in all printed works referring to this taxonomic segregate as a subspecies, from Fruhstorfer in ‘Seitz’ to Küppers in ‘Bauer’ (2006)—see synonymy above.

D'Abra (1985: 273) also proposed five subspecies for *C. myrina*, but not quite the same five as Tsukada (1985): *C. m. myrina*, *C. m. sarnada* and *C. m. vanbenmeleni* were the same, but he treated *C. m. ribbei* and *C. m. melancholica* differently and ambiguously. D'Abra's four images labelled as “*ribbei*” comprise two specimens from Peleng (*ribbei* sensu stricto), and

two from Palu (one indicated as a type). In his text he referred to all four under the heading “*myrina* ? subsp./? forma”, noting that the Palu material had “a close resemblance to the race from Banggai described as *ribbei* – which itself is probably only a d.s.f. [dry season form].” Finally, he included *C. m. melancholica* from eastern Sulawesi, which he described as “A large melanotic form, probably the w.s.f. of the eastern population of *myrina*.”

Examination of the Natural History Museum London (BMNH) collection reveals that D'Abra (1985) must have considered creating a new, separate subspecies for the Palu population, as the Palu specimen he figured as if it were a holotype is labelled as the “type” of an unpublished D'Abra manuscript name pinned in the Rothschild Collection. This specimen fits very well with *C. m. melancholica* from Palu and Palopo, as illustrated by Tsukada (1985), and as *melancholica* by Küppers (2006). However, according to Chris Müller (in litt., 2011), specimens he obtained from a collector at Palopo “are very orange”, not bluish, and so further work on the distribution and variation of *C. myrina* within the area currently considered to be occupied by subspecies *melancholica* (Fig. 1) may be needed.

Subject to the legal restrictions that apply to collecting this species, it would appear desirable to obtain fresh material from a range of localities for molecular work. In the process, any future reviser should be free to select the most appropriate syntype specimen, male or female, from Palopo or Mapane, as the name-bearing (lecto)type for *Cethosia myrina melancholica* Fruhstorfer, 1912.

Size: Males in BMNH have forewing lengths of 41.0–53.7 mm (N = 6, mean \pm 1 SD = 50.8 \pm 4.86 mm). No females available. On this slender evidence *C. m. melancholica* might be intermediate between *C. m. myrina* and *C. m. sarnada*, suggesting the further possibility that within the main island of Sulawesi there could be a north–south cline for decreasing size.

Cethosia myrina ribbei Honrath, 1887 (Fig. 9)

Cethosia myrina var. *ribbei* Honrath, 1887: 296, pl. 6, fig. 3 (male). INDONESIA: Sulawesi, Kep. Banggai [Peleng]. “Ins. Bangkai (östlich von Celebes)”. 12 male, 3 female syntypes. [At least two syntypes in BMNH; examined.]

Cethosia myrina ribbei Honrath: Fruhstorfer, 1912: 506; Tsukada, 1985: 77, 78, 296; D'Abra, 1985: 273 (as subspecies or dry season form); Vane-Wright & de Jong, 2003: 237; Küppers, 2006: 16, Pl. 17; Peggie, 2011: 16.

Note: the date of original publication for *ribbei* is

cited as 1886 in most recent publications, including Peggie (2011), but the correct date is 1887 (G. Lamas, pers. comm.).

C. myrina ribbei occupies the Banggai Archipelago (definitely recorded from Peleng and, apparently, Banggai Island), which lies about 20 km off the south coast of the central eastern peninsula of Sulawesi, close to its eastern tip. D'Abrera (1985) introduced a note of uncertainty regarding this subspecies, suggesting that it might represent nothing more than a dry season form of *C. m. melancholica* from the adjacent areas of eastern Sulawesi. However, although the two are similar in some respects, the colour pattern of *ribbei* is distinct and constant (notably the 'royal' blue colour of the male and, in both sexes, the large, half-moon shaped white mark in the centre of forewing cell CuA₂), and there seems no reason on present evidence not to regard the two as distinct.

If one accepts the BEAST/DIVA tree based on three data points presented by Müller & Beheregaray (2010: fig. 4), then *C. myrina ribbei* diverged from *C. myrina* in south-western Sulawesi (see discussion under *C. m. myrina* above) about 3–5 million years ago. Unfortunately there are no data concerning its possible divergence time from the eastern main-island population. To put a time-span of 3–5 million years into some context, according to May *et al.* (1995: 20), the "average lifespan of animal species in the fossil record, from origination to extinction, is around 10⁶–10⁷ years," while Ae (1988: 496) suggested that *Papilio* could achieve full species status after "one million years of almost perfect isolation." On this basis the present molecular evidence, although slight, is consistent with the hypothesis that this subspecies represents a distinct entity.

C. myrina ribbei is the smallest of the five subspecies (Küppers, 2006: 16). Honrath (1887) recorded the wingspan of 12 males as 79–82 mm, and of 3 females as 84 mm. Males in BMNH have forewing lengths of 39.7–49.2 mm (N = 6, mean \pm 1 SD = 46.53 \pm 3.50), while the single female available has fwl 50.0 mm exactly.

Cethosia myrina vanbemmeleni Jurriaanse & Lindemans, 1918 (Fig. 11)

Cethosia myrina bemmeleni [sic] Jurriaanse & Lindemans, 1918: 256. One male, one female syntypes, INDONESIA: Sulawesi, Buton (male: Boeton, xi.1916, N.H. Krans; female: Boeton, 1909, T. Elbert). [Presumed now to be in Naturalis, Leiden.] [Not examined.]

Cethosia myrina vanbemmeleni: Jurriaanse & Lindemans, 1920a: xlviii. [Short diagnosis; no



Figure 12. *Cethosia myrina vanbemmeleni*, Kakenauwe, Lambusango, Buton, Sulawesi Tenggara. Photographed by Nurul Winarni, and reproduced here with permission.

reference to 1918 description; one male only mentioned in text.]

Cethosia myrina vanbemmeleni [sic]: Jurriaanse & Lindemans, 1920b: 21. [Refers to 1918 description; one male only mentioned in text.]

Cethosia myrina vanbemmeleni Jurriaanse & Lindemans: Jurriaanse & Lindemans, 1920b: 88, pl. 4, two figures ('526': male, female).

Cethosia myrina vanbemmeleni Jurriaanse [sic]: Martin, 1921: 141.

Cethosia myrina vanbemmeleni Jurriaanse & Lindemans, 1918: Vane-Wright & de Jong, 2003: 237; Peggie, 2011: 16.

Cethosia myrina vanbemmeleni Jurriansz [sic]: D'Abrera, 1985: 273.

Cethosia myrina vanbemmeleni Jurriaanse [sic] & Lindemans, 1918: Tsukada, 1985: 77, 296; Küppers, 2006: 16.

Cethosia myrina vanbemmeleni [sic]: Küppers, 2006: pl. 18, figs 4–8.

Current usage of this name is based on Jurriaanse & Lindemans' subsequent spelling *vanbemmeleni* (the taxon was named after Professor J. F. van Bemmelen), which the authors introduced in two different publications in 1920 without an explicit justification for the change from their original 1918 spelling, *bemmeleni*. The 1920 spelling has however been universally applied. If we regard *vanbemmeleni* as an incorrect subsequent spelling in "prevailing usage" (ICZN, 1999: 121), then in accordance with Article 33.3.1 (ICZN, 1999: 43) and in the interests

of stability, the original authors' subsequent action and its adoption could be deemed to have created a correct original spelling. However, despite the lack of explanation for the spelling change that the original authors subsequently adopted, as suggested by Andrew Brower (pers. comm.), it appears better to regard this as an *emendation* rather than an incorrect subsequent spelling (very plausibly Professor van Bemmelen pointed out to the authors that 'van' was part of his surname and should be included). In which case, this being an unjustified emendation in prevailing use, it can now be deemed to be a justified emendation still bearing the original date and authorship under Article 33.2.3.1 (ICZN, 1999: 42).

Either way, I propose continuing acceptance of *Cethosia myrina vanbemmeleni* Jurriaanse & Lindemans, 1918, as the correct spelling, authority and date for the name applied to the Buton population of *C. myrina*. My justification is the statement of W.D.L. Ride: "In most cases an author will be required to maintain the particular spelling in prevailing usage for a name, even if it is found not to be the original spelling" (ICZN, 1999: xxviii). The name *C. m. bemmeleni* has never been employed by anyone in a printed work (other than *Zoological Record*) since its description, including the original authors who, for whatever reason, used *vanbemmeleni* instead in a follow-up note and a paper only two years later.

In passing, it should be noted that the authors' names have frequently been misquoted, the double- and double-a spelling of Jurriaanse causing particular difficulty.

Coloration: the almost obsolete white 'window' on

the hindwing upperside disc is the most notable feature of this subspecies together with, in museum material at least, the relatively uniform orange coloration of the upperside hindwing and posterior area of the forewing (Fig. 11). However, in living or very fresh material the posterior area of the forewing has a strong pinkish-magenta suffusion (Fig. 12), which fades soon after death – as often seen in the 'red' of *Heliconius* species, which can change from pinkish-red in living material to orange post mortem.

Size: the one male available in BMNH has a forewing length of 50.8 mm. The images in Tsukada (1985) and Küppers (2006) are consistent with the idea that this island race is larger than *C. m. ribbei*. All five subspecies can be separated using the key below.

Discussion: the ontological status of *myrina* subspecies

Despite periodic expressions of doubt about the nature and utility of subspecies (e.g. Wilson & Brown, 1953; Hennig, 1966: 46–57; Barrowclough, 1982; Vane-Wright & Tennent, 2011), most butterfly taxonomists willingly and often uncritically embrace the concept, with a seemingly endless flow of new subspecies still being described (see *Zoological Record* for the past 120 years). In practice the increasing availability of DNA data raises a growing number of empirical questions about the validity of many subspecies—many over-split, some apparently under (e.g. Tsao & Yeh, 2008; Makowsky *et al.*, 2010).

In the present case there has been little change in the division of *Cethosia myrina* into three subspecies on the main island of Sulawesi, together with two offshore

Key to subspecies of *Cethosia myrina*

The five subspecies of *Cethosia myrina* recognised here can be separated by the following key—although the phenotypic separation of the nominate subspecies and *C. m. sarnada* is exceptionally weak:

- 1 Upperside hindwing without pure white 'window' or patch covering base of cells R_5 and M_1 (which also extends to the distal part of the discal cell), this area at most slightly paler than the extensively orange or (very limited) purplish-brown discal and postdiscal areas of the wing (Fig. 11) (Buton, Muna) *C. m. vanbemmeleni* Jurriaanse & Lindemans, 1918
- Upperside hindwing with a clear white 'window' or patch covering base of cells R_5 and M_1 , extending into the distal area of the discal cell 2
- 2 Hindwing upperside of male with cells R_5 and M_1 distal to the white 'window,' and cells M_2 – CuA_2 extensively blue or violet, not brown or purplish brown 3
- Hindwing upperside of male with cells R_5 and M_1 distal to the white 'window,' and cells M_2 – CuA_2 distinctly tinged with or extensively coloured brown and / or purplish brown 4
- 3 Forewing conspicuously marked with clear white spots, most notably a large white postdiscal chevron-shaped mark in cell CuA_2 , which is at least 3.5 mm wide at its widest point; cells M_2 – CuA_2 in male extensively blue (Fig. 9) (Banggai Archipelago) *C. m. ribbei* Honnath, 1887
- Forewing not conspicuously marked with clear white spots, at most a small, usually rather indistinct, whitish spot or chevron-shaped mark in cell CuA_2 , at most 3 mm wide at its widest point and usually much smaller; cells M_2 – CuA_2 in male extensively violet (Fig. 8) (central regions of Sulawesi, from Palu to Palopo, eastern Sulawesi, and Togian islands) *C. m. melancholica* Frushstorfer, 1912
- 4 Larger subspecies (male forewing length 49–56 mm); in females veins crossing the white discal hindwing 'window' slightly more extensively marked by blackish scales (Figs. 2,3,6,7) (northern Sulawesi) *C. m. myrina* C. & R. Felder, 1867
- Smaller subspecies (male forewing length 42–54 mm); veins crossing the white discal hindwing 'window' of females slightly less extensively marked by blackish scales (Figs. 4,5,10) (south-western Sulawesi) *C. m. sarnada* Frushstorfer, 1912

island races, for almost a century. The only shift has been Küppers (2006) attempt to synonymise the northern and southern mainland subspecies as one, a decision reversed above on the evidence of size and the apparent division of the two by the central race. Is the traditional subdivision of this distinct species-level taxon justifiable in light of the continuing debate about the utility of subspecies?

Here, as in all areas of taxonomy, we run into problems of diverse underlying philosophy (Vane-Wright, 2001). In the case of subspecies, a non-obligate rank in the taxonomic system, there is currently a notable tension between a cladistic approach, with a desire for monophyly, and a purely empirical approach. According to the latter view, names for allopatric populations or groups of populations within a species that can be distinguished have heuristic value. If later research shows that such named subspecies are not useful, because they fail to capture or express anything of real biological significance, they can simply be synonymised without affecting the (obligatory) species name.

The problem with the cladistic or phylogenetic approach is that it can lead to taxonomic inflation (Isaac *et al.*, 2004); empiricism, on the other hand, readily brings about a proliferation of potentially meaningless subspecific taxa that can be misleading biologically, and may also devalue attempts to set meaningful conservation priorities. Such divisions have long been a bane of work on *Parnassius*, and now appear to be affecting, for example, *Ornithoptera*. In light of these concerns, how do the five subspecies of *Cethosia myrina*, a species protected under Indonesian law, compare?

In comparison to main-island *C. myrina*, both the offshore island populations are readily and reliably diagnosable on color and color pattern, as indicated above. Moreover, the existing DNA data suggest that *C. m. ribbei* (from Banggai Archipelago) diverged from at least one of the main-island populations several million years ago; no such data are available for *C. m. vanbemmeleni* (from Buton and Muna) but, given the striking change in color pattern, this may also represent a long-separate lineage. There would seem no grounds to synonymize these taxa with each other or any of the main island populations. From a phylogenetic or cladistic perspective they could well be full species—but to recognize them as such, at least at this stage in our understanding, would not appear helpful. On available evidence they are clearly peripheral representatives of the main-island taxon.

On the main island, were it not for the existence of the distinctive and somewhat *ribbei*-like central subspecies *C. m. melancholica*, from which *ribbei* would appear to have been derived, and which

apparently separates the main northern and southern populations, the case to synonymize *C. m. sarnada* with the nominate subspecies, as proposed by Küppers (2006), would seem strong—albeit with a possible south to north cline for increasing adult size. Thus the argument in favor of keeping *C. m. myrina* and *C. m. sarnada* separate is simply empirical—the hypothesis is that when other character systems are examined in depth (e.g. molecular characteristics) they will be found to differ. In this case maintaining the separate subspecies hypothesis is a stimulus to further research.

These differences in fundamental approach to recognizing subspecies even within a single polytypic species can only be justified on the basis that taxonomy is (inevitably?) a fractal pattern of doubt and certainty (Vane-Wright, 2003), a work forever in progress. This is particularly the case at the species level, which occurs at a notable boundary between pattern and process. We are invited to steer between Scylla and Charybdis: the multi-headed monster of taxonomic inflation on one hand, the whirlpool of excessive lumping on the other. Plus ça change? Simpson (1961: 110) suggested that, in some ways, classification should be seen as “a useful art”. When it comes to recognizing subspecies or not, perhaps he was right!

ACKNOWLEDGEMENTS

I am grateful to D. Peggie for drawing the ‘melancholia’ problem to my attention, pointing out ambiguities in the first draft, and suggesting valuable improvements and corrections to the final version. In completing the original MS I had generous help and critical discussion from my good friend Gerardo Lamas, notably concerning the dates of various publications, and interpretation of ICBN Article 33. Christian H. Schulze provided provenance data for one of two main-island specimens used by Müller & Beheregaray for DNA work; Chris Müller likewise gave additional information about the other. Sharon Touzel and Dez Mendoza (NHM London) and John Tennent kindly helped with references. Michael Boppré generously offered help with German translation. I am also very grateful to Keith Willmott for supplying images of *C. myrina vanbemmeleni*. Permission to reproduce the image included as Fig. 12 was kindly granted by Nurul Winarni. Fig. 1 is based on the map of Etsuzo Tsukada (1985: 296), which source is very gratefully acknowledged. Andrew Brower and two anonymous referees made a number of valuable comments and suggestions about the MS first submitted, leading to several clarifications and specific points that I have gratefully adopted. However, encouraged by Konrad Fiedler, the current MS has been considerably broadened in scope.

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Partitioning variation in duration of ant feeding bouts can offer insights into the palatability of insects: experiments on African fruit-feeding butterflies

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Abstract. Quantification of chemical defence contributes to the study of animal signals, and to understanding trade-offs among defences and life history traits. Some tropical fruit-feeding butterfly species can be expected to have well-developed anti-predator defences because they are long-lived, are host-plant specialists, and/or have contrasting colourations that may be involved in mimicry relationships. Yet, as a group they are often assumed to be palatable, even without supporting data. Palatability is a continuum that embraces within and between prey-species variation, and therefore, both among- and within-species variation must be documented. Palatability of nine species of fruit-feeding butterfly in Uganda was rated using a novel assay. One hundred and twenty-five butterflies were homogenized, their ground tissues suspended in sugar water and these suspensions offered as small droplets to individual ants in Petri dishes. The time ants spent feeding on these droplets was measured. Danaine butterflies were used as unpalatable references, and sugar solution as a palatable reference. Ants tended to eat in significantly shorter bouts from danaines compared to fruit-feeding species, and feeding bouts on pure sugar solution were longest. Within fruit-feeding species, variation in the duration of ants' feeding bouts was very substantial. There was also considerable variation among individual ants, such that large sample sizes would be needed to reliably distinguish palatability of different species of fruit-feeding butterflies. In explorative analyses, at least three fruit-feeding butterfly species that were assumed palatable appeared to be chemically defended. These results suggest that, in contrast to common assumptions, some tropical fruit-feeding butterflies use unpalatability for defence, perhaps contributing to their long life spans in the wild.

Key words: fruit-feeding, tropical, Nymphalidae, mimicry, colour, chemical defence

INTRODUCTION

Quantification of chemical defence is important for understanding the evolution of signals to predators, investments in other types of defences, and life history. Tropical fruit-feeding butterflies generally have long life spans, with many species

having longevity records that exceed six months (Kelson, 2008; Molleman *et al.*, 2007, F. Molleman unpublished data), therefore they must have effective anti-predator strategies. Chemical defence in long-lived butterflies in the tropics is demonstrated by several pollen-feeding butterfly species in the genus *Heliconius* that have long active life spans similar to fruit-feeding species (Ehrlich & Gilbert, 1973; Engler-Chaouat & Gilbert, 2007; Pasteels & Grégoire, 1983; Turner, 1971). Such association between chemical defence and long life spans in insects was proposed by Pasteels & Grégoire (1983), and butterflies that use more chemically defended host-plants live longer on average (Beck & Fiedler, 2009). However, in this latter multi-species comparison this effect was not statistically significant (Beck & Fiedler, 2009). One potential explanation for this could be that host-plant chemistry is a poor predictor of adult palatability given varying levels of sequestration and *de novo* synthesis, and thus chemical defence would be better measured using the butterflies themselves (Beck & Fiedler, 2009). Furthermore, many fruit-

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Received: 16 March 2012

Accepted: 5 June 2012

feeding butterflies are host-plant specialists (but not all, notable exceptions are grass feeding Satyrines and some *Charaxes*; e.g. DeVries, 1987; Larsen, 1991), a trait associated with the sequestration of defensive chemicals (Nishida, 2002). Moreover, chemically defended species often signal their unprofitability to predators with visual signals such as contrasting colour patterns (aposematism) and these signals can then be mimicked by other species that are not necessarily defended. Such contrasting patterns are found in many fruit-feeding butterfly species and mimicry does occur within this guild, but evidence for the nature of it (Batesian versus Müllerian, evasiveness versus palatability-mediated) is scarce. Therefore, it is likely that these colours function, at least in part, to signal unpalatability to predators.

Despite these reasons to suspect chemical defence in fruit-feeding butterflies, in the literature only a few species of fruit-feeding butterfly have been shown to be chemically defended (e.g. *Euphaedra cyparissa*: Larsen, 2007)—they are typically assumed to rely on evasive flight (Larsen, 1992b; van Someren & Jackson, 1959), crypsis, or eye-spots (Brakefield & Reitsma, 1991; Hill & Vaca, 2004; Marini-Filho & Martins, 2010) instead of chemical defence. It should be tested whether fruit-feeding butterflies that can putatively be classified as evasive (e.g. *Charaxes*, *Euphaedra*) and/or cryptic (e.g. *Kallimoides*, *Gnophodes*) also employ chemical defence in the form of unpalatability. Finally, insight into within-species variation in palatability is of interest. Various factors have been implicated in such variation, including age, sex, larval host-plant, and genetic differences in defence strategy (Alonso-Mejia & Brower, 1994; Brower *et al.*, 1982; Eggenberger *et al.*, 1992; Eggenberger & Rowellrahier, 1992, 1993; Holloway *et al.*, 1993; Moranz & Brower, 1998; Saporito *et al.*, 2010).

The present study used the duration of feeding bouts of workers of one ant species on butterfly suspensions as a measure of butterfly palatability. Warningly coloured species were hypothesized to be unpalatable, and chemical defence was hypothesized to play a role in an apparent mimicry relationship within the genus *Euphaedra*: *E. medon* Thurau 1903 females and *E. harpalyce* Talbot 1929 (not closely related within the genus). Mimicry in this group has been suspected to be based on signalling of unprofitability based on evasive flight (van Someren & Jackson, 1959), but strong evidence for such mimicry is lacking (Ruxton *et al.*, 2004). The technique used to measure palatability was convenient in a field setting under tropical conditions, provided values for individual butterflies, was free of prejudice based on any visual signal, was (nearly) independent of odour-mediated attractiveness, and subjects could be assayed over an extended period.

MATERIAL AND METHODS

Study site and subjects

This study was conducted at the Makerere University Biological Field Station in Kibale Forest National Park, Western Uganda. The field station borders selectively logged moist evergreen forest at an altitude of around 1500 m (Chapman *et al.*, 2005). Palatability tests were conducted on nine fruit-feeding butterfly species (Lepidoptera: Nymphalidae) illustrated in Fig. 1. Three species of danaines (Lepidoptera, Nymphalidae) were included (*Amauris niavius* (Linnaeus, 1758), *Tirumala petiverana* (Doubleday 1847), *T. formosa* (Godman 1880)) as examples of unpalatable butterflies (Jeffords *et al.*, 1979) including evidence for African species but not the particular species used (Larsen, 1983, 1992a, 2007). The fruit-feeding butterflies included species with brightly coloured and contrasting wing uppersides (*E. eusemoides* Grose-Smith & Kirby 1889, *E. alacris* Hecq 1979), one species with deep blue/violet females and metallic green males (*E. kakamega* van Someren 1934), and species with cryptic wing patterns (*Gnophodes chelys* Fabricius 1793, *Kallimoides rumia* Westwood 1850), as well as species that are neither particularly cryptic nor clearly warningly coloured (*E. medon*, *E. harpalyce*, *Harma theobene* Doubleday 1849, *Charaxes fulvescens* Aurivillius 1891). Larvae of most of these fruit-feeding butterflies are considered cryptic. However, *Euphaedra* caterpillars may be imprecise mimics of stinging slug-caterpillars (Limacodidae), and contrasting colours are found in the gregarious caterpillars of *E. kakamega* (black with light yellow bands: Molleman & Hecq, 2005) and to a lesser extent in those of *E. eusemoides* (green with dark dorsal setae: Molleman, in press). The combination of warning colours and gregariousness clearly indicates unpalatability (Sillen-Tullberg, 1988), and this is then usually transferred to the adult stage as well (Pasteels & Grégoire, 1983). The host-plants from which the studied species were reared in Kibale (Table 1) belong to families from which various unpalatable or toxic chemicals are known (e.g. Claudio *et al.*, 2009; Dongo *et al.*, 2009; Krief *et al.*, 2006; Penders & Delaude, 1994; Webber & Woodrow, 2009), except for the grass feeding *G. chelys*, while the host-plant of *K. rumia* is still unknown.

Experimental methods

Butterflies were either reared from field-collected caterpillars (most fruit-feeding butterfly individuals except *K. rumia*) or were collected from the field as

adults using sweep nets (danaines) or baited traps (all others). They were killed and the legs, wings, and head were removed before weighing. Each specimen was then ground up with three times its weight of boiled rainwater using a mortar and pestle. The resulting suspension was placed in a vial. In experiment I, a second solution was prepared in another vial that contained the same amount of water as was added to the butterfly suspension, and a ten percent sucrose solution was added to both vials so that both had a five percent sugar concentration. One droplet of each solution/suspension (one pair per Petri dish) was then placed two centimetres apart near the centre of a Petri dish (droplets were named 'butterfly' and 'sugar'). In experiment II, the droplet of sugar solution was omitted but the butterfly suspension was prepared in the same way.

Three ant species that were common in the vicinity were tested in preliminary trials, but only *Myrmicaria* c.f. *natalensis* Smith 1858 (subfamily Myrmicinae) workers walked around quietly and fed from the solutions offered, while the others tended to sit at the edge of the petri-dish without moving or ran about frantically. *M. natalensis* (Smith) (Hym: Formicidae) is a large, slow-moving, predaceous ant that forms large nests of up to several thousand workers (Arnold, 1924) and is very common around Kibale National Park. Worker ants from this species were collected from nearby ant trails and one ant was introduced into each Petri dish with a soft forceps. They were left in the Petri dish for up to 90 minutes, and were replaced after 15 minutes of inactivity. Even though ants may show different behavior in their natural context than when isolated in a Petri dish, workers of this species appeared to be reasonably at ease in our set up. Similar arena trials for example in bio-activity tests of larval sawfly haemolymph (Müller *et al.*, 2002) and for ranking the strength of interactions within and between species in the context of butterfly-ant mutualism (e.g. Ballmer & Pratt, 1991; Burghardt & Fiedler, 1996) have proven to be highly useful.

Observations were made by teams of two to five local technicians. Each person could simultaneously observe up to six Petri dishes, each containing one pair of droplets (or one droplet in experiment II) and one ant, while one person recorded the data. Start and end times for each ant feeding bout were noted in seconds. Local weather data were used to control for any temperature effect. Experiment I was first supervised by FM (May-June 2007), was then carried on without supervision, and was later supervised by MRW (July-August 2008). Experiment II was performed by local technicians without supervision (September 2008-July 2009).

Table 1. Host-plant information for the assayed butterflies in Kibale National Park, Uganda.

Butterfly species	Host-plant genus	family
<i>Danainae</i>	?	Asclepiadaceae, Euphorbiaceae
<i>Euphaedra kakamega</i>	<i>Aphania</i>	Sapindaceae
<i>Euphaedra alacris</i>	<i>Aphania</i>	Sapindaceae
<i>Euphaedra eusemoides</i>	<i>Uvariopsis</i>	Annonaceae
<i>Euphaedra harpalyce</i>	<i>Blighia, Aphania, Pancovia</i>	Sapindaceae
<i>Euphaedra medon</i>	<i>Paullinia</i>	Sapindaceae
<i>Harma theobene</i>	<i>Lindackeria</i>	Achariaceae
<i>Charaxes fulvescens</i>	<i>Allophylus</i>	Sapindaceae
<i>Kallimoides rumia</i>	?	
<i>Gnophodes chelys</i>	<i>Setaria</i>	Poaceae

Data analysis

Each ant's first choice of droplet (butterfly vs sugar) in experiment I was recorded. Ants typically returned to the same droplet but some switches were observed as well, and these were expressed as proportions. Statistical analyses were performed using linear mixed models on ant feeding bout durations in R (package lme4: Bates *et al.*, 2011), residuals showing an adequate fit of the modelling approach. Variation among individual ants and variation among butterfly individuals of the same species were captured with random effects, with ants nested in butterfly individual. We attempted to correct for possible differences among experiment days by using weather data as covariates for all data, and we used the duration of feeding bouts on sugar as daily references (for experiment I only). To determine whether a pooled analysis of data from experiments I and II was appropriate (the only difference between them being the presence/absence of the sugar droplet), we compared distributions of durations of two butterfly species that were well represented in both data sets, and compared species effect estimates between separate analyses of experiments I and II.

First, we tested whether fruit-feeding butterflies as a group could be distinguished from the references (danaines and sugar solution) using one-tailed tests. Second, we tested whether there were significant differences among the fruit-feeding butterflies using a two-tailed test. Lastly, effect estimates for butterfly species were calculated and compared to the references using one-tailed tests. To compare



Figure 1: Photographs of butterfly species on which palatability tests were conducted. **a.** *Euphaedra eusemioides* female. **b.** *Charaxes fulvescens* male. **c.** *E. alacris* female. **d.** *E. harpalycé* female. **e.** *E. medon* female. **f.** *E. kakamega* caterpillars. **g.** *E. kakamega* female. **h.** *E. kakamega* male. **i.** *E. medon* male. **j.** *Gnophodes chelys* female. **k.** *Harma theobene* male. **l.** *Kallimoides rumia* male.

durations of feeding bouts on butterfly suspension with those on sugar solution while using durations on sugar to correct for day effects, the feeding bout durations on sugar were subtracted from those on butterfly, and were then tested for equality with zero (data from experiment I only).

RESULTS

Experiment I yielded data from 57 butterflies (9 species), and experiment II from 68 butterflies (8 species) and the pooled data involved 663 feeding ants. The first choice of ants was biased towards

butterfly suspension for all species, including danainae. Switching occurred mostly from sugar to butterfly, except for danainae, *E. kakamega*, and *H. theobene* (Table 2).

Feeding bout durations were seemingly gamma distributed, ranging from a few seconds to about 10 minutes. Log transformation produced a normally distributed response variable that was used for all subsequent analyses and the graphical representation. Modelling log-transformed data using the normal distribution corresponds to the (untransformed) data being from the log-normal distribution. The interval for the mean feeding bout duration within a species could, therefore, be estimated while taking into account the residual variation in the model. For species that were well represented in both datasets, the distribution of durations of feeding bouts on butterfly droplets were very similar (Kolmogorov-Smirnov $D=0.11$, $p=0.42$ for *E. alacris* and $D=0.10$, $p=0.24$ for *C. fulvescens*). Moreover, the estimates for species effects were similar among the two experiments (Table 3). Therefore, it was justified to combine the data sets for this response variable.

Within-species variation was extensive (Fig. 2). Because the random effects are assumed to be normally distributed, we can analyze this variation in detail. For example, in the analysis of pooled data (Table 3), the standard deviation for the butterfly random effect was 0.54, meaning that for each species, individuals have a high probability of an average that

is up to 1.06 higher or lower than the average for the species, which is a lot because species estimates range between 3.23 and 4.35. The magnitude of the within-species variation can be illustrated further using *E. harpalyce* where the interval that includes about 95% of the individuals is 62 to 520 seconds. It was problematic to include butterfly age (freshly emerged vs field collected) into the model, because of unequal distribution among species and small sample sizes. Graphical representation did not suggest any correlation between age (freshly eclosed vs field collected) and palatability. For the species with the largest sample size (*C. fulvescens*) no significant age effect was detected either. Moreover, no sex effect was found in our data, and including our weather data did not improve our models. The variation among individual ants was also substantial with an SD for the pooled data of 0.37 (Table 3). No effect of order number of feeding of individual ants was detected.

The analysis of feeding bout durations on pure sugar showed that feeding bout durations differed among days, and a similar pattern was detected within the butterfly species such that there was a correlation between feeding bout durations on sugar and butterfly on particular days. However, daily maximum temperature was not correlated with ant feeding bout duration on sugar. Sugar solution was only used in experiment 1, and for these data correcting for such day effects by including durations on sugar as a co-factor improved the performance of

Figure 2. Palatability of butterfly species measured as duration of individual feeding bouts of ants (feedings) on butterfly suspensions (number of freshly emerged and field collected butterflies used). Box-plots represent averages among individual butterflies that are in turn based on varying numbers of feeding bouts with median, quartiles, and full range. The vertical dotted line represents the average duration of feeding bouts on sugar solution.

fresh/field # feedings

		Danainae
0/11	116	
26/4	448	<i>Charaxes fulvescens</i>
4/0	73	<i>Euphaedra kakamega</i>
13/5	287	<i>Euphaedra alacris</i>
5/1	76	<i>Euphaedra eusemioles</i>
3/6	189	<i>Euphaedra harpalyce</i>
4/13	385	<i>Euphaedra medon</i>
6/7	162	<i>Harma theobene</i>
0/9	136	<i>Kallimoides rumia</i>
6/2	121	<i>Gnophodes chelys</i>

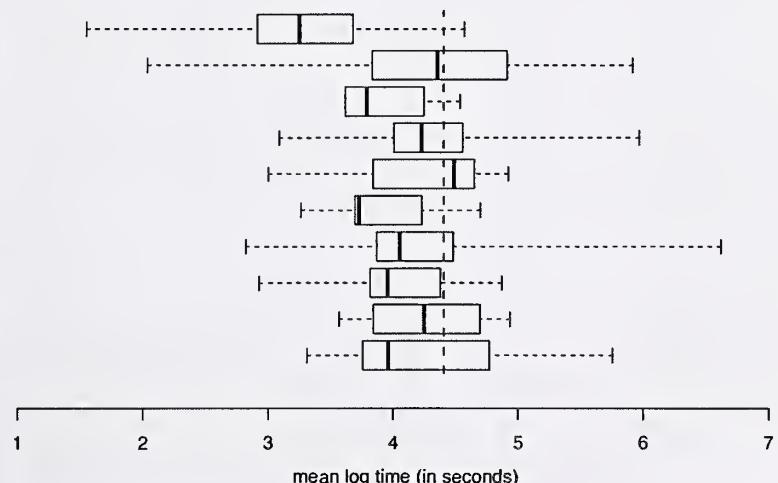


Table 2. Comparisons of ant feeding behaviour on sugar solution and butterfly suspension. For experiment I, choice behaviour is expressed as proportion of ants feeding first on butterfly or sugar solution, and subsequent number and proportion of switches. For feeding bout duration, parameter estimates for the linear mixed model of the log-duration of feeding bouts on butterfly suspension are reported first for experiment I, where for each observation day feeding bout durations on sugar were used as a reference, and second for the pooled data. P-values represent the equality with sugar in one-tailed tests without correction for multiple testing. As a group, fruit-feeding butterflies can be distinguished from sugar (random effects model, $P=0.021$). This table illustrates that 1) ants are more attracted to butterfly suspension than to pure sugar solution, and those ants starting with butterfly suspension are less likely to switch to sugar than vice versa, except in the case of three butterfly species that were also fed on for short feeding bouts; and 2) some butterfly species are fed on for similar feeding bout durations as sugar solution, while others appear less palatable to ants.

	Experiment I										Pooled data			
	1 st choice			Switch to			Feeding bout duration corrected for sugar				Distinguishing butterflies from sugar			
	N ants	Butterfly	switching	butterfly	sugar	N bouts	estimate	SE	p	N bouts	estimate	SE	p	
Danainae	13	0.69	2	0.00	0.22	27	-1.21	0.50	0.025	116	-1.17	0.22	<0.001	
<i>Euphaedra kakamega</i>	30	0.6	7	0.17	0.28	73	-0.05	0.35	0.878	73	-0.51	0.34	0.073	
<i>Euphaedra alacris</i>	49	0.84	10	0.25	0.20	114	0.07	0.24	0.774	287	-0.05	0.16	0.373	
<i>Euphaedra eusenoides</i>	43	0.67	8	0.43	0.07	76	0.08	0.29	0.790	76	-0.09	0.28	0.376	
<i>Euphaedra harpaluce</i>	18	0.83	4	0.67	0.13	38	-0.95	0.40	0.028	189	-0.55	0.21	0.009	
<i>Euphaedra medon</i>	14	0.79	4	0.67	0.18	30	0.77	0.48	0.128	385	-0.29	0.16	0.038	
<i>Harna thebene</i>	9	0.56	1	0.00	0.20	12	-0.49	0.58	0.409	162	-0.34	0.19	0.046	
<i>Charaxes fabriescens</i>	110	0.79	27	0.43	0.20	262	-0.3	0.16	0.083	448	-0.08	0.12	0.264	
<i>Kallimoides rumia</i>	31	0.81	6	0.50	0.12	66	-0.29	0.31	0.958	136	-0.10	0.23	0.398	
<i>Gnophodes thelys</i>	25	0.72	5	0.57	0.06	38	-0.35	0.36	0.346	121	-0.17	0.24	0.241	
SD (butterfly)							0.56				0.54			
SD (ants nested in butterfly)							0.53				0.37			
SD (residual)							1.18				1.27			

Table 3. Parameter estimates for linear mixed models of the log-duration of feeding bouts on butterfly suspension. P-values show the equality with danaines in one-tailed tests without correction for multiple testing. As a group, fruit-feeding butterflies can be distinguished from danaines (random effects model, $p<0.001$), and within this group species do not differ significantly from each other (random effects model excluding danaine and sugar data, $p=0.54$). N = number of feeding bouts, numbers of individual butterflies are given in Figure 2. N* is the estimated number of feeding bouts that needs to be measured to distinguish the butterfly species from danaines. This table illustrates that 1) results do not differ much between the two experiments so that pooling of data is justified, 2) random effects are large compared to species effect estimates, showing that there is important variation within species and among ants, 3) all of the fruit-feeding butterflies were fed on for longer bouts than the unpalatable danaines, *E. kakamega* being the only species that was not distinguished, which is attributable to an estimate near danaines as well as low sample size.

	Experiment 1			Experiment 2			Pooled data						
	N	Estimate	SE	p	N	Estimate	SE	p	N	Estimate	SE	p	N*
Danainae	27	2.29	0.54		89	3.53	0.21		116	3.23	0.22		
<i>Euphaedra kakamega</i>	73	3.91	0.39	0.012					73	3.89	0.34	0.058	81
<i>Euphaedra alacris</i>	114	4.48	0.26	0.001	173	4.24	0.17	0.008	287	4.35	0.16	<0.001	52
<i>Euphaedra eusemoides</i>	76	4.33	0.31	0.002					76	4.31	0.28	0.003	25
<i>Euphaedra harpalyce</i>	38	4.23	0.44	0.006	151	3.68	0.20	0.298	189	3.85	0.21	0.030	142
<i>Euphaedra medon</i>	30	4.36	0.53	0.007	355	4.06	0.13	0.020	385	4.11	0.16	0.002	111
<i>Harma theobore</i>	12	3.11	0.62	0.167	150	4.17	0.16	0.013	162	4.06	0.19	0.005	61
<i>Charaxes fultvescens</i>	262	4.51	0.18	<0.001	186	4.09	0.16	0.023	448	4.32	0.12	<0.001	73
<i>Kallimoides rumia</i>	66	4.53	0.33	0.001	70	3.94	0.30	0.138	136	4.30	0.23	0.002	37
<i>Gnophodes cheys</i>	38	4.61	0.39	0.001	83	3.92	0.25	0.120	121	4.23	0.24	0.003	39
SD (butterfly)		0.60				0.38				0.54			
SD (ants nested in butterfly)		0.46				0.29				0.37			
SD (residual)		1.21				1.31				1.27			

the statistical test. Nevertheless, pooled analyses for comparing fruit-feeding butterflies to danaines and to sugar solution remained preferred because of the larger sample size.

Ant feeding bouts were shortest for danaine specimens on average (Fig. 2), and as a group, fruit-feeding butterflies could be distinguished from them (random effects model, $p < 0.001$). On average, sugar was fed on for longer bouts than the fruit-feeding butterfly species combined (random effects model, $p = 0.021$). Some fruit-feeding butterfly species were fed on for shorter bouts than others on average (Fig. 2), but these differences were not significant (random effects model excluding danaine and sugar data, $p = 0.54$). In exploratory analyses (without correcting for multiple testing) *E. harpalyce*, *E. medon*, and *H. theobene* could be distinguished from sugar (Table 2), and *E. kakamega* could not be distinguished from danaines (Table 3). However, non-significance is attributable to low sample sizes, and estimates of the number of feeding bouts that need to be measured to statistically distinguish the species from the assayed danaines (N^*) may be best suited to preliminarily rank the species according to palatability. This suggests that *E. harpalyce* and *E. medon* are least palatable, followed by *E. kakamega* (Table 3).

DISCUSSION

We showed that certain African fruit-feeding butterflies can be moderately unpalatable to ants, and documented extensive variation in palatability within butterfly species. To interpret colour patterns and discover trade-offs with other defences and life history traits, it would be useful to know the palatability of butterflies to the relevant predators. However, observations of predation on fruit-feeding butterflies in the wild are extremely rare. If natural predators are visual hunting vertebrates (e.g. birds and lizards) as can be suspected, it can be hard to obtain palatability values for individual prey items that are unbiased by prey appearance and predator experience. Hence, assays with insects such as ants are useful (e.g. Eisner *et al.*, 2008). Given the diversity of potential predators in tropical forests, we expect that tropical butterflies that depend on unpalatability for survival should be distasteful to a wide range of predators, including ants. Moreover, congruence among predator species in their responses to defensive chemicals is usually substantial (Pasteels & Grégoire, 1983). For example, hornets, cats and humans ranked the taste of bird meat similarly (Cott, 1947). However, rarely is palatability of butterflies rated using more than one potential predator (Trigo, 2000), and differences in

type of chemical defence most effective to different classes of predators have been noted as well. For example, *Paederus* beetle larvae produce pederin as a defense against spiders, which is not effective against other arthropods (Kellner & Dettner, 1996). While realizing the need for further tests, we interpret shorter feeding bouts of ants as indicative of lower palatability to most generalist predators.

The first droplet that ants fed on was usually butterfly suspension, which may represent a preference or simply an effect of detectability as the butterfly suspension may emit a stronger odour. Therefore, cafeteria experiments where ants choose between food sources do not measure only palatability, but also detectability. Ants' switching behaviour was consistent with the results of feeding bout durations: butterfly species that were fed on for short feeding bouts were also more often switched away from.

The duration of ant feeding bouts varied considerably for individual butterflies of the same species and for individual ants. However, in our data we did not find within-species correlations between ant feeding bout durations and butterfly age or sex. Apart from variation in defensive chemistry, variation in nutritional value (depending on reproductive history and nutritional status of the individual butterfly) could also have contributed to this within-species variation in palatability albeit mainly in the minority of butterflies that had been field-captured. Ant behaviour could vary according to local climate, lineage-specific traits, as well as nutritional status of the colony or individual (e.g. whether an individual was going out to forage or was coming back with food). However, the nutritional status of individual ants did not appear to affect feeding bout duration because the order number of feeding of individual ants did not affect it.

When feeding bout durations on sugar droplets could be used as reference for observation days, some of this variation could be accounted for, leading to greater power for distinguishing butterfly species. This suggests that day-to-day variation in our data is mainly caused by weather, despite lack of correlation with the daily maximum temperature.

Based on the duration of ant feeding bouts, fruit-feeding butterflies are on average more palatable than danaines. As a group fruit-feeding butterflies could be distinguished from the danaines, and all except *E. kakamega* were distinguishable in the exploratory analysis. The interpretation of tests against the pure sugar solution is less straightforward because the sugar solution offers only sugar and water, while the butterfly suspension offers the ants the same sugar concentration, but with added nutrients as well as

defensive chemicals from the butterfly. Thus, the value of a sugar control may lay primarily in its role in accounting for variation in ant behaviour. While, as a group, fruit-feeding butterflies were fed on for significantly shorter bouts than pure sugar solution, differences for several species were small and only three fruit-feeding species (*E. harpalyce*, *E. medon*, and *H. theobene*) were distinguished in the exploratory analysis.

Results of the exploratory analyses are biologically interpretable. The similarity between *E. medon* females and *E. harpalyce* may be an example of Müllerian mimicry, because both species were less palatable than sugar solution and were the hardest to distinguish from Danaines (N* 142 and 111 feedings, respectively). *E. kakamega* also appeared to be unpalatable, and this is not surprising because of its warningly coloured gregarious caterpillars. The adult dorsal wing colouration is also non-cryptic and may be mimicked by other species, most notably *E. uganda* (Aurivillius 1895). *H. theobene* was also distinguished from sugar in the exploratory analyses but appeared more easily distinguished from danaines. Any chemical defence in this species may be related to the suspected sequestration of host-plant chemicals (probably cyanides) in this group (van Velzen *et al.*, 2007), but is surprising in the light of its rather cryptic appearance. This may demonstrate that chemical defence is not always advertised with contrasting colour patterns (Endler & Mappes, 2004). We have noted adverse reactions to other cryptic fruit-feeding butterflies (Molleman *et al.* 2010) but this did not bear out in our analyses of feeding bout durations. On the other hand, contrastingly coloured species *E. alacris* and *E. eusemoides* did not appear to be particularly unpalatable, and these are more likely Batesian mimics of chemically defended moths. However, all such hypotheses on particular species generated by our exploratory analyses need to be tested with further palatability assays, and, most critically, observations on avoidance behaviour of potential predators such as birds and chameleons. Nevertheless, our results indicate that one cannot assume that fruit-feeding butterflies are all equally palatable, despite strongly developed evasive flight and crypsis in this group.

Obtaining palatability data needed to elucidate the evolution of defence and signals such as colour patterns, and their relationship to life history evolution is challenging. It is important to distinguish between tests that measure (innate or learned) responses to appearance, odour, palatability, toxicity, or a combination of these. Moreover, variation within species may also be extensive and of biological

interest, and therefore, assays that produce palatability estimates for individuals are preferred over those that yield only a population mean with confidence interval. Given that there is a gradient rather than a dichotomy of palatability (Brower *et al.*, 1968), continuous measures such as feeding bout durations more readily provide statistically significant results. Using common omnivorous and easy-to-handle ants, we presented such a method, that is convenient in a field-lab setting (also in tropical regions) and requires little training.

However, we suggest several improvements to the method presented. Responses of animals used in assays vary over time and among individuals and lineages, and this can be countered by; 1) using positive and negative controls with each test, preferably for each ant; 2) using worker ants from several documented colonies; 3) only picking individuals that leave the colony on a foraging trail; and 4) recording local conditions (e.g. temperature and humidity for each assay). While a sugar solution is a straightforward palatable control, a more standard negative control would be preferable. If a freezer is available, a stock suspension made from a large number of known unpalatable insects could serve as such. However, to compare palatability among different regions, a global standard of 'mixed defensive chemicals', needs to be developed. Animals behave differently towards potential food in a natural setting than when isolated in a Petri dish. With video cameras ant feeding bouts could be recorded in cafeteria experiments in a natural setting. It can be expected that a much wider range of ant species would be amenable for such approach. This is important when attempting to measure feeding bout durations where such docile ants are not available, and also to test for congruence in the responses of multiple ant species. Moreover, with video images it would be easier to code transient behaviours such as running away from food and grooming, and to distinguish between active feeding and resting at the food. While it remains to be shown that other ant species also adapt their feeding bout durations according to palatability of the food, we believe that this parameter is a relatively efficient metric for ranking the palatability of animals.

ACKNOWLEDGEMENTS

We thank Boniface Balyeganira, Harriet Kesiime, Christopher Aliganyira, Francis Katuramu Kanywanii, John Koojo, Francis Akoch Edigu, Małgorzata E. Arlet and Mary Aluin, for their invaluable assistance in the field and in the laboratory. Weather data were kindly provided by Colin and Lauren Chapman. We thank Torben Larsen for sending us important literature, Phil Ward for identifying our ant samples and Johanna Mappes,

Elizabeth Long, Philip DeVries, Konrad Fiedler, Annette Aiello, Stephen Malcolm, James Fordyce, Torben Larsen, Toomas Tammaru, Siiri-Lii Sandre and anonymous Smithsonian Tropical Research Institute staff members for insightful discussion and critical comments. We thank the Uganda Wildlife Authority (U.W.A.) and the Ugandan National Council for Science and Technology (U.N.C.S.T.) for permission to carry out the research. Funding for the field work was provided by the National Institute on Aging (PO1 AG022500-01 and PO1 AG608761-10 to JRC) and University of California, Davis (Bixby International Travel Grant to MRW), and analysis and write up were supported by Estonian Science Foundation grants 7406, 7699, 7522, 8413 and GD6019, targeted financing project SF0180122s08 and by the European Union through the European Regional Development Fund (Centre of Excellence FIBIR).

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Survival patterns under Costa Rican field conditions of the gregarious caterpillar *Euselasia chrysippe* (Lepidoptera: Riodinidae), a potential biological control agent of *Miconia calvescens* (Melastomataceae) in Hawaii

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Abstract. Survival of *Euselasia chrysippe*, a natural herbivore of *Miconia calvescens*, was investigated under field and laboratory conditions in Costa Rica as part of a biological control program in Hawaii. Following its introduction to Pacific islands, *M. calvescens* has become a dominant invasive species and the gregarious caterpillar of *E. chrysippe* has emerged as a promising biological control candidate. Under laboratory conditions, survivorship from egg to adult was 63%. *E. chrysippe* produced viable clutches in an experimental field plot. Similar clutch sizes occurred in both natural habitats and the experimental plot. Stage-specific life tables encompassing two years in the experimental plot indicate that larval survivorship from egg to the end of the sixth instar was about 10%. Egg parasitism was low in natural habitats and nil under experimental conditions. Larval group sizes were similar in natural and experimental field conditions, suggesting that survivorship is similar in both environments. During the coldest dry period no larvae survived past the fifth instar. Rainfall was a limiting factor for the survivorship of *E. chrysippe* in the experimental plot, but temperature appears to be the factor that would limit the effect of *E. chrysippe* on *M. calvescens* in Hawaii. Efforts should be invested in natural quarantine facilities to provide a testing ground for this species in target environments.

Keywords: Classical biological control, clutch size, *Euselasia chrysippe*, gregarious caterpillar, larval survivorship, *Miconia calvescens*, out-planting

INTRODUCTION

Miconia calvescens D.C. (Melastomataceae) is a dominant invasive species in Hawaii, Australia and some tropical oceanic islands of the South Pacific. It is native to the Americas, from southern Mexico to northern Argentina (Medeiros & Loope, 1997). Following its introduction into Hawaii as an ornamental in 1961, it invaded a wide variety of habitats, including agricultural landscapes as well as

wet forests, and was declared a noxious weed in 1992 (Kaiser, 2006). Several methods have been tested for its eradication, including aerial spraying of herbicides and manual extraction, but the large seed banks (Loope, 1997; Medeiros & Loope, 1997) make these methods costly and ineffective. To protect native ecosystems on tropical Pacific islands the use of introduced biological control agents is an option, which could prove to be not only safer, but also more effective than chemical applications (Denslow & Johnson, 2006).

Selection of the best agents for biological control is critical for this kind of program to achieve success. Many programs have failed due to the lack of basic information on the environmental requirements of candidate species (Hokkanen, 1985; Wapshere *et al.*, 1989) and poor climatic adaptation is the main reason given for failure in classical biological control programs worldwide (Stiling, 1993). Due to underlying biophysical factors, climate influences the establishment and survival of candidate biological control agents (Hoelmer & Kirk, 2005).

Biological control agents should originate from areas having a similar climate to that of the intended area of release (Stiling, 1993). A program to search

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Received: 27 December 2011

Accepted: 29 July 2012

for biological control agents of *Miconia calvescens* in Costa Rica at the University of Costa Rica (UCR) began in 1999, and since then more than 50 species of phytophagous insects have been collected from this plant species (Hanson *et al.*, 2009). Among them, *Euselasia chrysippe* (Lepidoptera: Riodinidae) emerged as a viable candidate (Johnson, 2009), but detailed knowledge of its biology is necessary to evaluate the suitability of this species as a potential biological control agent. *E. chrysippe* ranges from southern Mexico to Colombia (DeVries, 1997; Warren *et al.*, 2005) and occurs commonly from sea level to 1500 m. In Costa Rica it is found on both the Caribbean and Pacific slopes in primary and secondary rain forests (DeVries, 1997). *Miconia elata* (DeVries *et al.*, 1992), *M. calvescens* (DeVries, 1997), *M. trinervia*, *Conostegia rufescens* (Melastomataceae) (Janzen & Hallwachs, 2005), *Miconia impetiolaris*, *M. appendiculata*, *M. donaena* and *M. longifolia* (Nishida, 2010) have been reported as host plants for *E. chrysippe*. The caterpillars of *E. chrysippe* are processionary and synchronize their movement, feeding, resting and molting (DeVries, 1997; Allen, 2010; Nishida, 2010).

Classical biological control projects have shown that understanding the response of the biological agents to various environmental conditions increased the chances of success (Caltagirone, 1981). Larval mortality factors and general life history of *Euselasia* species have only been investigated in depth in Brazil, where several species are defoliators of *Eucalyptus* (Zanuncio *et al.*, 1990, 2009; Zanuncio *et al.*, 1995; Sousa *et al.*, 2010). The primary objectives of this study were to generate life tables for the pre-adult stages of *E. chrysippe* on *M. calvescens*, compile information on their environmental requirements, and identify the main sources and trends of pre-adult mortality in natural habitats and an experimental field plot. The out-planting (experimental field) strategy has been infrequently used in the context of biological control, but when it has been used, it has provided positive results (Balciunas *et al.*, 1994; Wilker *et al.*, 2000). The present study seeks to provide background information on the climatic suitability of *E. chrysippe* before its release in Hawaii.

MATERIALS AND METHODS

Field data and specimen collection

Between 2003 and 2005 egg clutches and larvae were collected from the following sites in Costa Rica: Laguna Hule (10°18'15" N, 84°12'23" W; 750 m), Lake Arenal (10°28'17" N, 84°46'11" W; 525 m), El Ángel-Cariblanco (10°15'44.2" N, 84°10'19" W; 750 m), La

Selva Biological Station (10°25'52" N, 84°00'12" W; 50 m), Vereh (9°47' N, 83°31' W; 1200 m), Jabillos (9°54' N, 83°37' W; 750 m), Jicotea (9°48'29" N, 83°31'23" W; 900 m), Hitoy Cerere Biological Station (9°40'19" N, 83°01'28" W; 100 m), Las Cruces Biological Station (8°47'28" N, 82°57'26" W; 1095 m), and Cerro Nara (9°30' N; 84°01'2 W; 1000 m). In the Holdridge life zone classification system (Holdridge, 1967) all sites in this study are either tropical wet or rain forests, from basal to lower montane elevational zones. Each site was visited between one and seven times, and the eggs and larvae were taken from *Miconia calvescens* trees or saplings. Not all individuals were collected, since some clutches and larvae were just photographed (using a Nikon Coolpix 4500 camera) for data on clutch and group sizes in their natural habitats. Causes of egg mortality in the field (predators, parasitoids or pathogens) were determined visually on site.

Larval rearing in the laboratory and the experimental plot

Clutches (attached to a piece of leaf) were placed in Petri dishes, larvae in plastic bags (on leaves), and transported to the laboratory at the UCR in San José (elevation: 1100 m). In the laboratory, egg clutches were left in the Petri dishes until eclosion. Larvae were placed on *M. calvescens* potted plants, one clutch per plant, and pots were placed in large plastic containers half full of water to prevent escape. After the larvae pupated on the plants, the pots were placed in a controlled environmental chamber (Model 518, Electro-Tech Systems: 23°C, 14:10 h D/L, 70% RH). Larvae were monitored daily; digital photographs were taken every day to determine the exact number of individuals. This procedure was also followed when eggs brought from the field hatched. Larval survivorship and instar duration were thus obtained for *E. chrysippe* under laboratory conditions. Laboratory temperature was recorded using a HOBO® datalogger.

Adults were released into the Leonel Oviedo Biological Reserve (experimental field plot) close to *M. calvescens* trees. Twenty *M. calvescens* trees were planted in the reserve between July 2002 and March 2003 to form a small patch. By September 2003 the average height, DBH and number of leaves of the saplings were 1.7m, 21mm, and 23 leaves, respectively. By November 2006 the trees had grown to 4.1m, 57mm and 76 leaves. The Leonel Oviedo Biological Reserve is a 0.75-hectare secondary forest fragment located in the middle of the UCR campus in San José (9°56'15"N; 84°03'00"W) completely immersed in an urban environment (Nishida *et al.*, 2009). This patch served as an experimental field, as neither *E. chrysippe*

nor *M. calvescens* are native to this part of the country. This area corresponds to Tropical Premontane Moist Forest (Holdridge, 1967), characterized by a dry season with little or no rain between December and April.

After adults had been released, daily surveys of *M. calvescens* trees in the experimental plot were carried out to determine when and where the egg clutches were laid. As in the laboratory, digital photographs were taken to quantify eggs per clutch, and after hatching photographs were taken of each larval group to count the number of larvae left and to determine the changes in larval stage. These data were used to calculate pre-adult mortality for each cohort (each individual egg clutch), for all stages. Sixth instar larvae abandon their plant and pupate, so it was impossible to determine pupal mortality in the experimental plot.

Survivorship of immature stages in the experimental plot

Time periods were established based on weather. The study extended for 20 months and encompassed two rainy and two dry seasons. The four periods were: 1. 8 September to 30 December 2003 (Rainy 2003); 2. 19 December 2003 to 29 March 2004 (Dry 2004); 3. 28 July to 15 November 2004 (Rainy 2004); and 4. 29 December 2004 to 2 April 2005 (Dry 2005). Developmental periods extended from the day an egg clutch was laid to the day the last larva died or left the *M. calvescens* tree. The duration of the egg stage was compared between periods using analysis of variance (ANOVA); in this and subsequent ANOVA tests a Tukey post-hoc test was used to localize differences between means. The same was done with maximum daily temperature and minimum daily temperature; total precipitation was also quantified for each period (obtained from a meteorological station 200m away from the experimental plot). Weather data from La Selva Biological Station (1987-2006) and Las Cruces Biological Station (1994-1998, 2005, 2006) (OTS 2012) were used to establish a temperature range for this butterfly species in the wild.

For larval survivorship, all data were converted to stage-specific survivorship (the proportion of each cohort that entered the population as an egg and survived through each instar). The rate of larval survivorship was compared between periods, performing log-log (base 10) regressions on the proportion that survived per stage versus stage (survivorship curves). The slope for each cohort was calculated (Hunter 2000) and the means of the slopes were compared between stages (Rainy 2003, Dry 2004, Rainy 2004, Dry 2005) with an ANOVA.

RESULTS

Egg clutch and larval group sizes of *Euselasia chrysippe* in the field and experimental plot

Between September 2003 and January 2005, 690 adult females and 570 adult males raised in the laboratory were released in the experimental plot (Fig. 1). There was a bias of 18.3 females (SD = 8.1; N = 29) compared with 15.8 males (SD = 8.4; N = 29) ($t_p = 2.84$; df = 29; $P = 0.01$). A total of 149 egg clutches were laid on the leaves of *Miconia calvescens* trees in the experimental plot. Most of the clutches were laid within 3 weeks, 25 days being the longest period between release and finding of a clutch. Monthly egg clutch production was correlated neither with adult release ($r = 0.02$; $n = 19$; $P = 0.94$; Fig. 1) nor precipitation ($r = 0.07$; $n = 19$; $P = 0.79$; Fig. 1).

In the field (10 sites in Costa Rica) the mean number of eggs per clutch was 62.7 (SD = 22.3, $n = 84$, range: 13 to 134) and followed a normal distribution (Shapiro-Wilk's test: $SW-W = 0.98$; $P = 0.4$; Fig. 2). In the experimental plot the mean was 67.2 (SD = 19.4, $n = 158$, range: 15 to 131), also fitting to a normal distribution ($SW-W = 0.99$; $P = 0.25$; Fig. 2). There was no significant difference in clutch sizes between field and experimental plot ($t = -1.61$; df = 240; $P = 0.11$). The condition of clutches in the field was in general good; fifteen healthy clutches were found and collected, another 61.5% ($N = 78$) had all empty eggs showing that larvae had eclosed successfully. Only 19.2% ($N = 78$) of the egg clutches had damaged

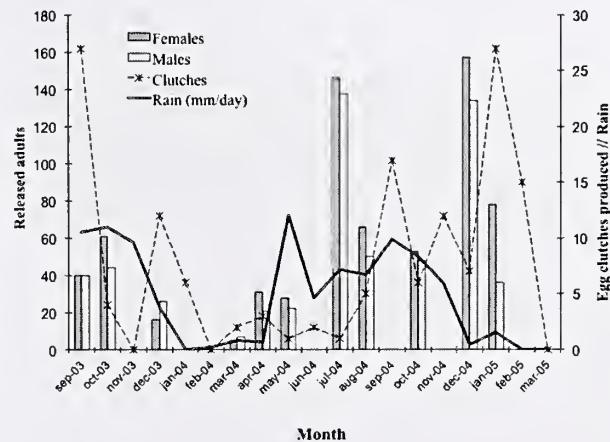


Figure 1. Release of adult *Euselasia chrysippe* and their egg clutch production in the Leonel Oviedo Biological Reserve (UCR; experimental field plot). The black line represents mean daily precipitation per month.

eggs, 11 (14.1%) of these had been attacked by parasitoids. There was no evidence of egg parasitoids in the experimental plot.

For some instars, a higher number of larvae per group were found in the field than in the experimental plot. There were more larvae per group in the second and third instars (Table 1), but not for the first, fourth and fifth instars; very few sixth instar groups were found in the field.

Survivorship of immature stages

In the experimental plot hatching time was longer in the Dry 2005 period (from 25 to 43 days) than in other periods ($F_{3,56} = 4.72; P = 0.005$; Table 2). For the other three periods combined, the duration of the egg stage lasted between 21 and 37 days. Table 2 shows the comparison of environmental factors during all periods. The maximum daily temperature was lower during the Dry 2005 period ($F_{3,406} = 12.4; P < 0.0001$), as was the minimum daily temperature ($F_{3,406} = 9.3; P < 0.0001$). The annual pattern of daily temperature range in the experimental plot is similar to that found at Las Cruces BS, but much lower when compared to La Selva BS (Fig. 3). During the rainy periods rainfall was more than 300 mm/month, but less than 16 mm/month during the dry periods (Fig. 1). In its natural habitats *M. calvescens* and *E. chrysippe* experience a maximum of one month without rain (Fig. 3).

The number of eggs per cohort did not differ between periods in the experimental plot ($F_{3,88} = 2.3; P = 0.90$; Table 3). During the Rainy 2003 period there was a clutch where all eggs failed to hatch; in the Dry 2004 period two leaves with clutches attached dried out and fell. During the Dry 2005 period half of the cohorts were

Table 1. Mean (\pm SD) number of *Euselasia chrysippe* larvae per group by instar, found on *Miconia calvescens* trees in the field (10 sites in Costa Rica) between December 2003 and August 2005, and in the Leonel Oviedo Biological Reserve (experimental field plot) between October 2003 and April 2005.

Instar	Field	n	Reserve	n	t	P
First	42.4 ± 22.5	12	37.2 ± 20.1	68	0.82	0.42
Second	47.1 ± 20.9	20	34.9 ± 21.0	39	2.11	0.039
Third	43.6 ± 22.7	15	29.5 ± 14.7	34	2.6	0.01
Fourth	29.7 ± 18.6	9	27.6 ± 13.8	29	0.36	0.72
Fifth	22.5 ± 31.4	6	24.0 ± 13.8	25	0.07	0.94
Sixth	7.0 ± 8.5	2	21.4 ± 14.1	19	^a	

^a test was not performed due to small sample size.

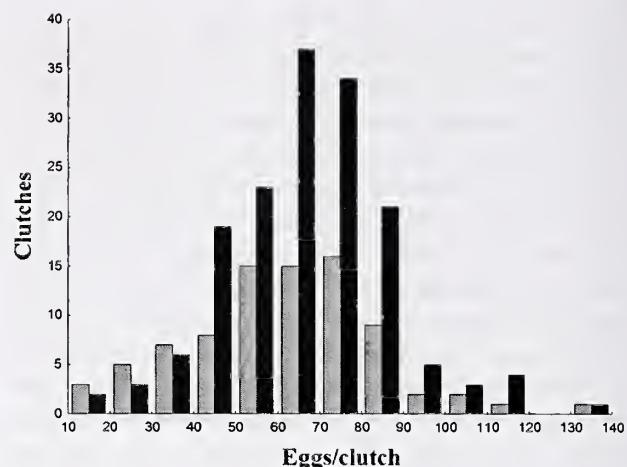


Figure 2. Number of eggs per clutch of *Euselasia chrysippe* found on *Miconia calvescens* trees. Black bars represent clutches found in the Leonel Oviedo Biological Reserve (experimental field plot) between October 2003 and December 2005; gray bars represent clutches found in the field (10 sites in Costa Rica) between December 2003 and December 2005.

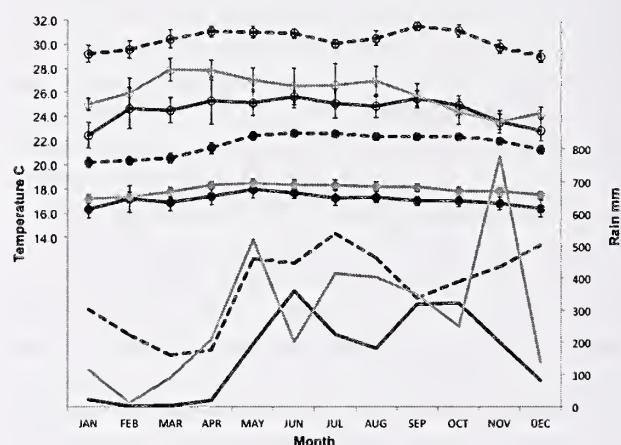


Figure 3. Mean (\pm SD) monthly minimum (full circles) and maximum (empty circles) temperatures ($^{\circ}$ C; left Y-axis), and total monthly precipitation (no circles; right Y-axis), for two sites in Costa Rica where *Miconia calvescens* grows naturally and the Leonel Oviedo Biological Reserve (experimental field plot – black continuous line). La Selva BS (black discontinued line) is the hottest and Las Cruces BS (gray line) is the coldest (and driest) sites where *M. calvescens* and *Euselasia chrysippe* occur together in Costa Rica.

lost during the egg stage (Table 3): five fell with leaves, three were preyed upon by ants, three did not hatch, in two cases the larvae hatched but died next to the egg shells (with no evidence of predation), and in three cases the larvae hatched but disappeared without feeding.

It was more likely for early instar larvae to die during the dry periods than during the rainy periods ($F_{3,88} = 7.91$; $P < 0.0001$; Fig. 4). The number of cohorts ($X^2 = 3.51$; $df = 2$; $P = 0.17$) and larvae per cohort ($F_{2,16} = 0.54$; $P = 0.59$) did not differ between the Rainy 2003, Dry 2004 and Rainy 2004 periods (Table 3), through the end of the sixth instar. All fifth instar larvae died during the Dry 2005 period (Fig. 4). For the other three periods larval survivorship to the end of the sixth instar was about 10%. Mortality due to egg predation (by ants) occurred during all periods, but overall predation rates could not be quantified under experimental conditions or in their natural habitats.

The number of survivors in the laboratory decreased slowly and constantly until the end of the sixth instar (Fig. 4), reaching 63.1% ($SD = 7.1$, $N = 7$) from egg to adult. No cohort reached 100% mortality in the laboratory. The duration of the entire larval period was shorter in the laboratory, with a mean of 36.5 days ($SD = 3.8$), compared to 46.6 days ($SD = 5.0$) in the experimental plot ($t = 4.9$; $df = 24$; $P < 0.0001$). Mean temperature was higher in the laboratory than in the experimental plot, 22.2°C ($SD = 1.0$) versus 20.2°C ($SD = 1.2$) ($t = -5.5$; $df = 260$; $P < 0.0001$).

DISCUSSION

Euselasia chrysippe survived successfully in an experimental field plot, under less than ideal biotic and abiotic conditions. Adult females reared in the laboratory mated and laid eggs on *Miconia calvescens* trees, even though the latter were smaller than in natural habitats (Badenes-Pérez *et al.*, 2010). Egg clutch and larval group sizes of *E. chrysippe* found under experimental conditions reflected what was found in the field. A general larval survivorship (experimental plot) of 10% to the pupal stage agrees with reports for other gregarious species of Lepidoptera and Symphyta (Hunter, 2000). Survivorship was lower in the first two larval instars, especially during the dryer periods, which is also common in other Lepidoptera (Zalucki *et al.*, 2002).

Egg parasitism of *E. chrysippe* was low in the field and similar to that found by Nishida (2010), much lower than the parasitism rate for *E. eucerus* eggs in Brazil (Zanuncio *et al.*, 2009). The lack of evidence for parasitoid mortality in the experimental plot probably reflects the isolated location of this small patch of secondary forest. The closest fragment of forest is 4.3 km away, and the closest old growth forest is 12 km away (Braulio Carrillo National Park). An egg parasitoid of *E. chrysippe*, *Encarsia porteri* (Aphelinidae), has been reared in its native range (Nishida, 2010). Although many *Encarsia* species have been introduced to Hawaii to control whiteflies (Heu *et al.*, 2004), *E. porteri* is not among them (Bishop Museum, 2002). This has major implications for the introduction of *E. chrysippe* in Hawaii, as it reduces the risk of egg parasitism should it be released as a biological control agent of *M. calvescens*. Larval group sizes observed in the field versus the experimental plot remained similar until the end of the fifth instar. Few sixth instar groups were found in the field, and these groups were small compared to the ones in the experimental plot. One likely mortality factor acting on these older larvae in the field are tachinid parasitoids, as reported by Janzen & Hallwachs (2005) and Nishida (2010). Larval parasitism and predation were not quantified in this study, but predation events were commonly observed in all larval stages in the experimental plot.

Low precipitation and temperatures were correlated with high mortality and local extinction of immature stages of *E. chrysippe* under experimental field conditions. This long dry season can be considered extreme for this species, since in its native range in Costa Rica there is typically a mean rainfall of 280-600 mm/month (Fig. 3; Allen, 2007) and a maximum of one dry month. The absence of rain

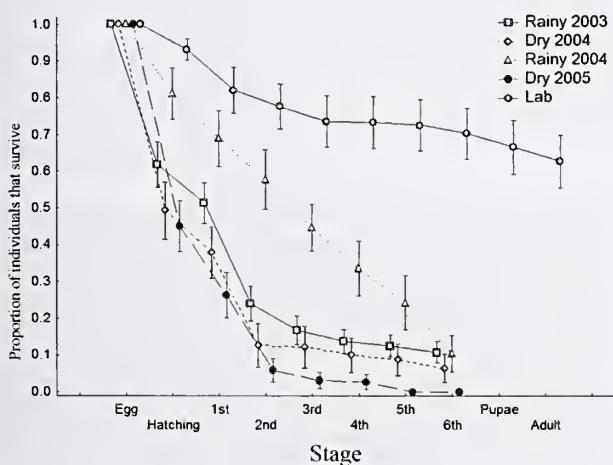


Figure 4. Survivorship (Mean \pm SE) of *Euselasia chrysippe* individuals on *Miconia calvescens* trees in the Leonel Oviedo Biological Reserve (experimental field plot) during four time periods and in the laboratory (UCR). Slope (Mean \pm SE) of the regression between survivorship and larval stage: -0.53 ± 0.12^{ab} (Rainy 2003; $n = 26$), -0.80 ± 0.15^{bc} (Dry 2004; $n = 19$), -0.26 ± 0.06^a (Rainy 2004; $n = 16$) and -1.10 ± 0.12^c (Dry 2005; $n = 31$). Means followed by the same letter are not significantly different ($P > 0.05$, Tukey test).

Table 2. Duration (Mean \pm SD) of the egg stage for *Euselasia chrysippe* during four time periods in the Leonel Oviedo Biological Reserve (experimental field plot), and the recorded environmental factors associated with each period.

Variable	Rainy 2003	Dry 2004	Rainy 2004	Dry 2005
n (egg clutches)	23	13	7	17
Duration of egg stage (days)	30.1 \pm 3.5 ^a	29.8 \pm 4.3 ^a	29.0 \pm 2.3 ^a	34.1 \pm 5.0 ^b
Environmental factor				
Maximum Daily Temperature (°C)	24.8 \pm 1.8 ^a	24.4 \pm 2.6 ^a	25.0 \pm 1.5 ^a	23.2 \pm 2.8 ^b
MaxDT range (°C)	19.0-28.1	19.8-30.9	19.9-27.9	17.8-29.9
Minimum Daily Temperature (°C)	17.0 \pm 0.8 ^a	17.3 \pm 1.8 ^a	17.0 \pm 0.8 ^a	16.4 \pm 1.4 ^b
MinDT range (°C)	15.0-18.8	13.0-22.6	14.5-19.1	13.3-19.0
n (days)	102	102	111	95

Means followed by the same letter within a line are not significantly different (P > 0.05, Tukey test).

Table 3. Partial life table for the immature stages of *Euselasia chrysippe* on *Miconia calvescens* in the Leonel Oviedo Biological Reserve (experimental field plot) during four time periods. Numbers represent quantity of cohorts that had living individuals at the end of each stage.

Variable	Rainy 2003	Dry 2004	Rainy 2004	Dry 2005
Clutches (cohorts)	26	19	16	31
Eggs/cohort ^a	67.3 \pm 23.4	62.4 \pm 11.3	64.8 \pm 14.0	65.3 \pm 23.3
1st instar (cohorts)	23	15	15	15
2nd instar	16	4	15	4
3rd instar	13	4	15	4
4th instar	13	4	10	2
5th instar	13	4	8	.
6th instar ^b	12	3	4	.
Larvae/cohort 6th instar ^a	19.0 \pm 15.2	28.3 \pm 17.9	23.3 \pm 8.1	.

^a Mean \pm SD, there is no difference between periods (P > 0.05, ANOVA); ^b there is no difference between periods (P > 0.05, X²).

for more than four months in the experimental plot caused *Miconia calvescens* leaves to dry out and fall from the trees, causing the immediate loss of many egg clutches. The areas of *M. calvescens* infestations in Hawaii do not suffer from a dry season longer than a month (Giambelluca *et. al.*, 1986), thus probably reducing to a minimum this kind of mortality (T. Johnson, pers. comm.). Daily temperature range in the experimental plot is similar to that at Las Cruces BS, the coldest site where both *M. calvescens* and *E. chrysippe* occur in Costa Rica (Allen, 2007); however, during the Dry 2005 period the daily temperature was at least 1°C lower than during the Dry 2004 period. This could explain the zero survivorship during the coldest dry period, as it seems that a mean minimum daily temperature of 17.0°C is the lower thermal limit for *E. chrysippe* in natural habitats (Fig. 3). If introduced to Hawaii, *E. chrysippe* would probably not

be able to establish in all areas where *M. calvescens* is invasive, as this plant has invaded areas with minimum daily temperature reaching 10°C in both Hawaii (Allen, 2007) and Tahiti (Meyer, 1998). Eggs took longer to hatch during the coldest dry season in the experimental plot. It remains to be seen if this had a negative effect on larval survivorship, but eggs were definitely exposed longer to predators, leaf fall and also suffered an increased risk of dehydration and depletion of food reserves (Holliday, 1985).

This study shows that larval survivorship of *Euselasia chrysippe* in an experimental field plot is similar to that in natural habitats and the chief limiting factor for pre-adult survivorship is a four-month dry season. The areas of *M. calvescens* infestations in Hawaii have a favorable rainfall of 150-300mm/month (Giambelluca *et. al.*, 1986; Juvic & Juvic, 1998; Reichert *et al.*, 2010), with no dry season longer than a month.

With respect to temperature, the areas most affected by *M. calvescens* fall into the range tolerated by *E. chrysippe* (Allen, 2007; Kaiser, 2006), but some of the less affected are too cold. This might be a non-issue since early detection of *M. calvescens* in the island of Kauai (Kauai Invasive Species Committee), which is too cold for *E. chrysippe*, has prevented its expansion and there might not be any trees of reproductive age on the island (Conant & Nagai, 1998). These results are encouraging for the possible introduction of *E. chrysippe* to Hawaii as a biological control agent of *Miconia calvescens*.

The current levels of scientific review have notably increased the safety of biological control programs in Hawaii since 1975 (Reimer, 2002); no purposely introduced species approved for release have been recorded to attack native or other desirable species in the last 37 years (Funasaki *et al.*, 1988; Reimer, 2002). There is thus good precedent for continuing this process with *E. chrysippe*. The present study provides essential data on general survivorship patterns and climate suitability; the next steps will be to quantify the impact of predation and parasitism on the larval stages, examine host specificity in greater detail, and determine the probability of in-situ establishment. Probable biotic sources of mortality of *E. chrysippe* larvae in Hawaii include generalist predators (Johnson, 2009) such as introduced vespid wasps and ants (Gambino *et al.*, 1987; Reimer, 1994). Finally, efforts should be made to provide adequate space for butterfly mating in the quarantine facilities in Hawaii, and field cage trials to provide suitable testing grounds for this species in target environments.

ACKNOWLEDGEMENTS

The Universidad de Costa Rica and the state of Hawaii, Department of Land and Natural Resources, USGS Biological Resources Division and the National Park Service, via the University of Hawaii Cooperative Studies Unit, and the USDA Forest Service International Programs, supported this research. I also thank the *Miconia* project staff for their help in the field and for logistical support; Paul Hanson, Johel Chaves-Campos, William Eberhard, Gilbert Barrantes, Sarah Graves and two anonymous reviewers for comments on the manuscript, and Tracy Johnson and Edgar Rojas for making the *Miconia* project possible.

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NOTE

**First record of the Lime Swallowtail *Papilio demoleus* Linnaeus, 1758
(Lepidoptera, Papilionidae) in Europe**

Papilio demoleus (Linnaeus, 1758), the Lime Swallowtail, is one of the most widespread members of the family Papilionidae and one of the most studied butterfly species partly due to the quick expansion of its range and potential danger to agriculture (Eastwood *et al.*, 2006; Goyle, 1990; Mérit *et al.*, 2009; Smith & Vane-Wright, 2008; Wehling *et al.*, 2006; Zakharov *et al.*, 2004). This Indo-Australian species originally had a strongly disjunct distribution. The first part ranged from the Arabian Peninsula in the west through tropical Asia to Okinawa (Japan) in the east (nominate subspecies), and mainland Southeast Asia in the south (ssp. *malayanus* Wallace, 1865). The second part of the range encompassed Australia (ssp. *sthenelus* Macleay, 1826), some of the Lesser Sunda Islands (ssp. *sthenelinus* Rothschild, 1895), and southern Papua New Guinea (ssp. *novoguineensis* Rothschild, 1908). The latter three subspecies are well differentiated in DNA, morphology, and life history (Smith & Vane-Wright, 2008; Zakharov *et al.*, 2004). Most importantly, whereas *Citrus* trees and other members of the Rutaceae family constitute the main hostplants of the Asian populations, the caterpillars in the Australian region only feed on Fabaceae (*Cullen*), and thus have never been noted as a pest on *Citrus* (Fenner & Lindgren, 1974; Smith & Vane-Wright, 2008; Straatman, 1962; but see Tripathi *et al.*, 1998). No populations were known from the remaining Indo-Australian archipelago, until the species started its expansion into the Philippines in the late 1950s (Smith & Vane-Wright, 2008). During the 1980s and 1990s, *P. demoleus* continued its range expansion across Indonesia, including the Lesser Sunda Islands which were already inhabited by ssp. *sthenelinus* (Matsumoto, 1996; 2002; Moonen, 1991;

1999; Rawlins, 2007; Smith & Vane-Wright, 2008), and reached northern Papua New Guinea in 2004 (Tennent *et al.*, 2011; Wiemers, 2007).

At its western range margin, *P. demoleus* has also extended its range in historical times from Iran into Iraq and around the Persian gulf, following the cultivation of *Citrus* (Larsen, 1977; 1984). In Oman, it occurs now in close proximity to its Afrotropical relative *Papilio demodocus* Esper, 1799, which also feeds on *Citrus* (Clarke *et al.*, 1963; Henning *et al.*, 1997; Larsen & Larsen, 1980). Most recently, *P. demoleus* extended its range further into the Palaearctic Region. In 2003 and 2004, *P. demoleus* appeared in central Syria, near Al Qaryatayn and Palmyra, and shortly afterwards, in 2005, it was recorded for the first time in Turkey, where populations were established in city parks and gardens with lemon trees in Nusaybin city (Mardin Province) close to the Syrian border (Benyamin *et al.*, 2007; Koçak *et al.*, 2006; Koçak & Akdeniz, 2008).

At about the same time, the species was discovered in the New World, where the ssp. *malayanus* was accidentally introduced to the Caribbean (Eastwood *et al.*, 2006). It was first recorded in 2004 from the Dominican Republic on Hispaniola (Guerrero *et al.*, 2004), and since then has spread quickly to the neighboring islands of Puerto Rico, Jamaica, and Cuba (Garraway *et al.*, 2009; Homziak & Homziak, 2006; Lauranzón Meléndez *et al.*, 2011).

Apart from its first appearance in the Caribbean region, most of the range expansion of *Papilio demoleus* appears to be due to immigration and subsequent assimilation at new territories, following corridors of *Citrus* plantations everywhere. Even though this species usually displays stationary behavior, its migratory capabilities are well known from occasional regional migrations which can involve thousands of individuals (Dell, 1977; Dingle *et al.*, 1999; Ramesh *et al.*, 2012; Smithers & McArtney, 1970). The range expansion in the Indo-Australian archipelago was possibly facilitated by deforestation coupled with increased cultivation of *Citrus* and international trade (Smith & Vane-Wright, 2008).

Since the records of the species in Syria and Turkey in 2003-2006, no further range expansion into the Palaearctic region was observed. Until now,

Received: 9 August 2012

Accepted: 27 August 2012

the record near Al Qaryatayn in Syria ($34^{\circ} 13'N$, $37^{\circ} 14'E$) has been known as the westernmost population of the species in Eurasia. This is most interesting, because this Syrian location is a mere 120 km from the Mediterranean Sea, and Nusaybin in Turkey already belongs to the Mediterranean Region, which is one of the largest *Citrus*-producing areas in the world.

On 10 April 2012, the first author found a *P. demoleus* male in Portugal, Algarve, Município Loulé, south of Loulé, in the vicinity of Dos Quartos ($37^{\circ} 06'33''N$, $8^{\circ} 02'18''W$). The butterfly was flying in the *Citrus* garden of the villa Quinta Mimosa (Fig. 1). No previous record is known from Portugal or any other European country (Kudrna *et al.*, 2011; Maravalhas, 2003). The first record of this species in Europe, in the western Palaearctic region, is particularly interesting and important, taking into consideration the great distance of more than 4000 km to the previous westernmost locations in Syria (Fig. 2). A natural immigration to the Algarve appears most unlikely due to the long distance to the closest populations and the only minor wing damage of the specimen (but see Fraser, 1946). Furthermore, the collected specimen appears to belong to the subspecies *malayanus* according to wing pattern characters and not to the nominate subspecies which is found in SW Asia. Instead, the species was probably introduced with cultivated *Citrus* or by international trade. In the case of the introduction to the Caribbean it has been speculated that *P. demoleus* was imported for a release at a wedding ceremony, by hobbyists, or that it escaped from a butterfly house (Benyamini *et al.*, 2007; Wehling *et al.*, 2006). As far as we know, there are no butterfly houses in the Algarve region, but some exist in the Lisbon area, at a distance of about 200 km. A genetic study of the European specimen is planned to help reveal its origin.

Further monitoring of the area is necessary to verify whether *P. demoleus* is established in Portugal. It is possible that this record remains a singularity, as was the case with the single specimens of the Afrotropical *Papilio demodocus* which have been found in California (USA) and East Timor during the 1960s (Mendes & Bivar de Sousa, 2010; Tilden, 1968). In April 2012 adverse weather conditions (rains and strong wind) did not allow further observations. A permanent occurrence is not excluded because of the abundance of larval host plants there and suitable climatic conditions. Although the climate is slightly cooler than in Turkey and Syria (with mean annual temperatures of $17.4^{\circ}C$ in Faro compared to $18.8^{\circ}C$ in Al Qamishli, Syria), the winters in the Algarve area are warmer (with average minimum daily temperatures per month of $7.3^{\circ}C$ compared to $2.3^{\circ}C$ in Al Qamishli; Fig. 3).



Figure 1. Male of *Papilio demoleus*: Portugal: Algarve: Faro: Loulé: Dos Quartos, 12 April 2012, D. V. Morgan leg.

Until recently, *Papilio demoleus* was thought to be a species confined to warm climates, and that a climatic barrier was limiting its further northward expansion from the Arabian Peninsula into the Mediterranean Region. The recent records indicate that *P. demoleus* might be a more adaptive species which can acclimatize in regions with slightly different conditions and colonize the *Citrus* plantations there. Some authors associate the *P. demoleus* range extension with recent climatic warming in the Northern Hemisphere that move the climatic borders previously limiting the species' distribution (Benyamini *et al.*, 2007). In Nusaybin (Turkey), *P. demoleus* was able to survive the winter 2005/2006, even though temperatures dropped below $0^{\circ}C$ on two days in February (to $-2^{\circ}C$). Compared to the reference period 1961-1990, however, the winter 2005/06 was $2.4^{\circ}C$ warmer (Fig. 4), and it remains to be seen if *P. demoleus* populations can survive here. The following winters (apart from the even warmer winter 2009/10) had mean temperatures close to the long-term average. In Syria, the species seems to be well established, as it was recorded again in 2007 and 2010, both times at Dayr Az Zawr (data obtained from www.observado.org). This location is along the Euphrates River, halfway between Palmyra and Nusaybin, and has a very similar climate, especially regarding minimum temperatures in winter (20 annual frost days compared to 16 frost days in Nusaybin).

If *P. demoleus* is able to survive in areas with Mediterranean climate, this could have serious implications for the *Citrus* industry in the

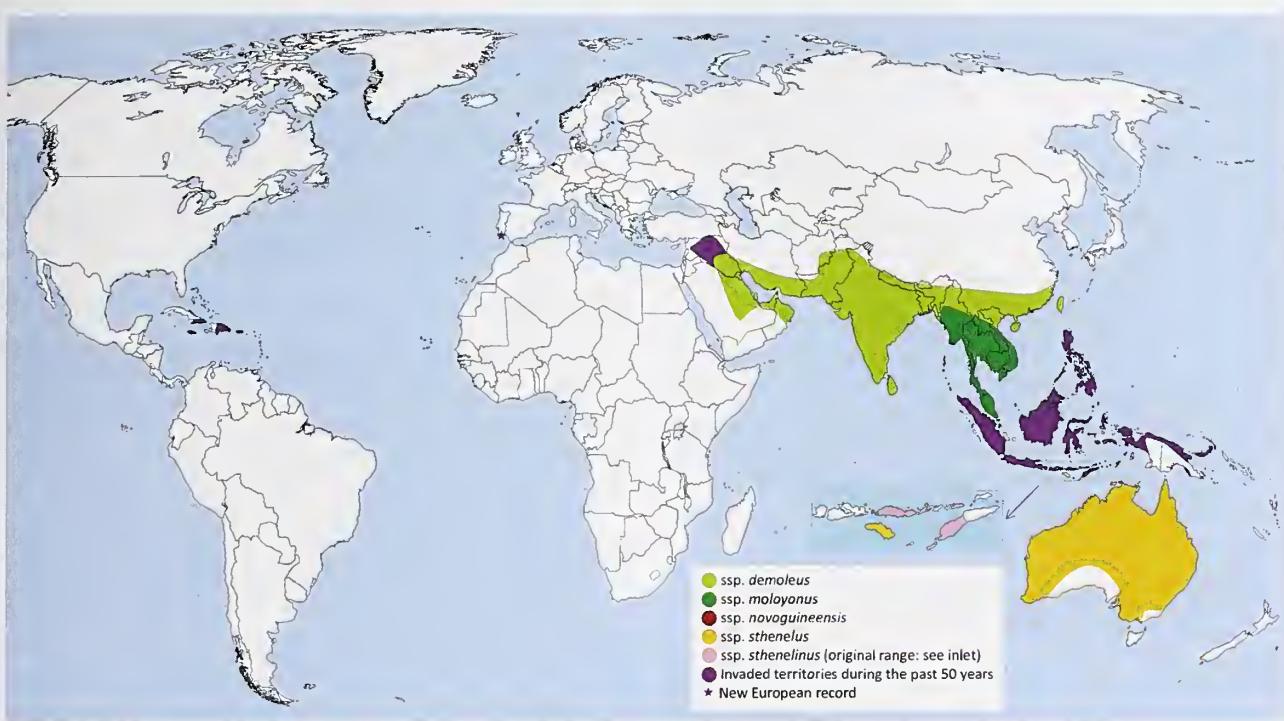


Figure 2. Distribution of *Papilio demoleus*. The inset shows the distribution of *Papilio demoleus* in the Lesser Sunda Islands before the invasion from SE Asia (i.e. 1990). Last records of ssp. *sthenelinus* currently known to us are from 1988 (Alor), 1992 (Timor), and 1997 (Komodo). First records of invasive populations in the Lesser Sunda Islands are from 1990 (Sumbawa), 1991 (Lombok), 1993 (Timor), 1995 (Leti), 1997 (Flores), 2002 (Wetar), and 2007 (Alor).

Mediterranean region. Larvae of *P. demoleus* can entirely defoliate orange and lemon trees, especially young trees, and populations can proliferate and disperse quickly. In most of its range the species has 4-6 generations per year. In Iran, the complete life cycle only takes about 33-35 days (Abivardi, 2001).

An example of another *Citrus*-feeding lepidopteran species with South Asian origin which has greatly expanded its range in recent decades is the Citrus Leafminer, *Phyllocnistis citrella* Stainton, 1856 (Gracillariidae). It is now a pest in all major *Citrus*-growing areas of the world, including the Mediterranean region, most of Africa, the Americas, New Guinea and Australia (De Prins & De Prins, 2012; EPPO, 2012; Hoy & Nguyen, 1997; Waterhouse, 1998). It even managed to reach many remote islands and archipelagos such as Guam, Samoa, Mauritius (1995), Micronesia (1996), the Azores (1997), Bermuda (1998), Hawaii (2000), and Galapagos (2005). *P. citrella* spread with a remarkable speed: Since its discovery in Florida in 1993, it took only 3 years for its expansion into most American countries from the Southern USA via Central America and the Caribbean to Argentina, and during the same time period all Mediterranean countries were colonized as well.

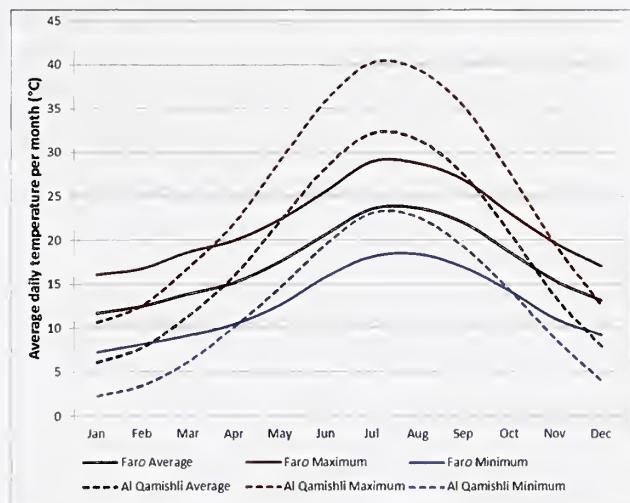


Figure 3. Average daily temperatures per month in Faro (Portugal) and Al Qamishli (Syria) in the period 1961-1990.

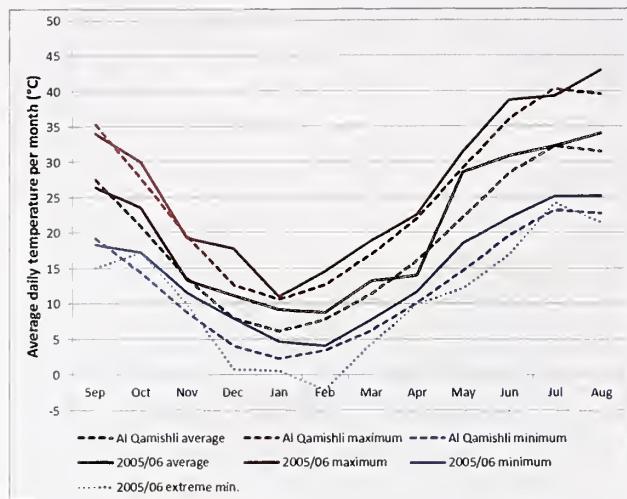


Figure 4. Average daily temperatures per month in Al Qamishli (Syria) in 2005/06 and in the period 1961-1990.

The Mediterranean area is the only major *Citrus*-growing region in the world which is not yet inhabited by a *Citrus*-feeding *Papilio* species. Therefore it would be potentially important to know whether *Papilio demoleus* is able to establish here. The only major *Citrus* production areas which have not yet been invaded by *P. demoleus* are the Afrotropical Region, which is already inhabited by *Papilio demodocus*, and the American continents, where *Citrus* is grown from California and Texas (USA) in the north to Brazil (the world's largest producers of oranges), Uruguay and Argentina in the south. However, the expansion of *P. demoleus* to the Americas from the Caribbean will probably happen during the next few decades.

ACKNOWLEDGEMENTS

We thank two anonymous reviewers for their comments which helped to improve the paper.

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BOOK REVIEW

***Lepidoptera Argentina, Parte I: Castniidae* by F. C. Penco, 2011**

Distributed by the author. Morón (Argentina), 41 pp. ISBN 978-987-33-0384-5. Price: approx. US\$ 25.00

South America is the continent that harbors by far the highest species richness of all the Lepidoptera. Only a few recent book series aim at covering some larger fraction of country-wide faunas beyond the butterflies. One example is the series *Mariposas del Ecuador*, edited by Francisco Piñas Rubio and colleagues between 1997 and 2006 – which, however, contains little more than photographs of specimens (frequently in poor quality and without proper identification) plus their collection data. The slender volume discussed here is the first part of a planned 20 volume series that intends to cover the fauna of a large, though for the most part non-tropical, Latin American country, viz. Argentina. From the subtitle it becomes clear that these books will not (and cannot) be full 'scientific monographs'. Rather they shall provide illustrated and commented catalogues that render the fauna accessible to further and more detailed study. It is this perspective that I have taken to review the first issue that has now been published.

This initial volume deals with the Castniidae, a small family of little more than 200 species worldwide. Nine-teen of these have been recorded in Argentina, and two more species are illustrated as they may occur (or might have historically occurred) in the country. Thus, the book figures about 15% of Neotropical Castniidae diversity – not a bad start for a moth family that most lepidopterists will be rather unfamiliar with. To start such a book series with a small and comparatively 'easy' family (in terms of taxonomy) was certainly a good idea. Overall this little volume is quite convincing. Following a short general prelude and an illustrated introduction into the family, the main part of the book is comprised of the species accounts. Each species is illustrated

in color, usually both sexes and often also in dorsal and ventral view. The species accounts are short; they consist of a list of synonymous names under which each species has been treated thus far, a brief description of their geographic distribution (overall and within Argentina), a small distribution map, a paragraph on their known larval host-plants, and supplementary information. A references list and an index conclude the volume.

I found the photographs of good (though not excellent) quality. What appeared to be most useful for a reader (like myself) rooted in evolutionary biology, ecology and biodiversity research, were the distribution maps and the host-plant records. I was surprised to learn that only four castniid species have more substantial geographic distributions within Argentina, whereas the remainder is confined to the tropical north-eastern tip (province of Misiones) and thus are certainly endangered by the massive urban development and environmental change going on around Buenos Aires. I was also impressed that for quite a number of species at least some basic data on larval host-plant affiliations are available – which were carefully extracted from various (often historical) sources. An odd old record of an Apiaceae host-plant for *Geyeria uruguayana* seems highly improbable, though, and might better have been qualified as such.

There are a few critical issues to remark on. First, I doubt whether it is necessary (and useful) to illustrate so few moths per page. For a family of modest diversity this may be acceptable, but for more speciose taxa it would certainly be helpful to have the plates more densely used with figures (enabling direct comparisons across various species). Scale-bars should also be included with the figures, and information on characters that are important for identification (either in the text or by indication on the plates) will be valuable with more species-rich and taxonomically more complicated taxa. Second, including at least a brief summary for each species in English would render the book series far more accessible to the non-Spanish speaking world (the species accounts themselves are not problematic in that regard). Third, the references need to be more carefully cross-checked between the text body and

Received: 3 September 2012

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the references list. I stumbled over a sizeable number of citations in the text which are unfortunately not included in the references list. To give just one example for a species that is nowadays of peculiar concern in Europe as an introduced pest of palm trees, *Paysandisia archon*: of 12 literature sources cited in its species account, six are not found in the references list. Moreover, quite a number of papers from the last decade on this species of economic interest were unfortunately not covered as well. The recent synopsis of Castniidae from Paraguay (Ríos & González, 2011) probably appeared too late in print to be included in the reviewed book.

Overall, however, this book is a promising start to a series on the Lepidoptera of Argentina. I wish this series a healthy development and very much look forward to seeing additional volumes to appear in print.

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Observations on the life history and field biology of an imperiled butterfly *Philotiella leona* (Lepidoptera: Lycaenidae) from South Central Oregon

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Abstract. Observations on the life history and field biology of the highly range-restricted south-central Oregon butterfly, *Philotiella leona* Hammond and McCorkle (Lepidoptera: Lycaenidae), are presented. Oviposition was difficult to obtain in captivity. Eggs were laid on or adjacent to flower buds of the larval host Spurry buckwheat, *Eriogonum spargulinum* (Polygonaceae) and hatch in 4-5 days at 25-27°C. Larvae feed only on flower buds and flowers, have four instars and reach the prepupal stage after 10-12 days at 25-27°C. Pupae are formed after a further 5-6 days and overwinter. Adults fly from mid June to the end of July, males eclosing a few days before females. Mating and oviposition occur soon after eclosion and the sex ratio is relatively balanced through most of the flight period. Flight is meandering and low to the ground, most common after mid morning when sunny and temperatures are > 21 °C. Nectaring occurs on at least six species of flowering plants.

Keywords: *Philotiella leona*, endangered species, life history, biology, *Eriogonum spargulinum*

INTRODUCTION

Leona's little blue butterfly, *Philotiella leona* Hammond and McCorkle, is arguably the most range-restricted and endangered butterfly species in the United States. Discovered in 1995, *P. leona* is restricted to less than 32 km² in the Antelope Desert of south central Oregon (Hammond & McCorkle, 2000; Pyle, 2002; Warren, 2005; Miller & Hammond, 2007; Ross, 2008; 2009; Matheson *et al.*, 2010). It appears to be a highly specialized species occupying a volcanic ash and pumice ecosystem using the desert-restricted annual Spurry buckwheat, *Eriogonum spargulinum* A. Gray (Polygonaceae) as its larval host. *Eriogonum spargulinum* occurs commonly in similar desert habitats in California, Nevada and Idaho (Goodman, 1948). *Philotiella leona* is currently being considered for listing under the Endangered Species Act (Matheson

et al., 2010). Apart from brief and fragmentary notes presented by Hammond & McCorkle (2000), Ross (2008, 2009) and Matheson *et al.* (2010), little is known of the biology or life history of *P. leona*. This paper provides images and information on the development of immature stages of *P. leona* as well as field observations on adult biology.

MATERIALS AND METHODS

Immature stages. *Philotiella leona* was reared in the laboratory from gravid females, eggs and larvae collected from the Antelope Desert in Klamath County, Oregon during June-July 2011. On July 1, seven females showing oviposition behavior were obtained, stored in plastic containers in a cool box (10-15°C) and transported to Prosser, WA. A second group of 20 females was obtained on July 7 and a third group (n = 8) on July 25. Seven eggs and one fourth instar larva were collected on July 16. Flowering stems of *E. spargulinum* were collected randomly from one (~0.5 ha) location on July 24 and 29 and stored in three plastic containers (34 x 24 x 12 cm) on each date. Containers were held at 25-27 °C and inspected daily for wandering *P. leona* larvae. Larvae were collected, measured (to identify instar) and reared (see below). Gravid females were held individually or in groups of 4-12 in small, cylindrical plastic containers (12 x 13 or 23 x 13 cm) with gauze lids for oviposition. Host

Received: 11 April 2012

Accepted: 5 October 2012

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plants (*E. spurgulinum*) with flower buds (July 1) or flowers (July 7, 25) were provided in containers as cut stems in water or as potted plants. Nectar was provided by *E. spurgulinum* flowers and supplemented with cut flowers of *Achillea millefolium* L. Paper towel pads soaked in dilute sucrose solution were provided for additional nourishment. Most oviposition cages were held indoors at 25-30°C under natural day lengths and exposed to sunshine for at least 8 hours/day. Some cages were held under fluorescent lighting (natural day lengths) at 35-39°C (day) and 25°C (night). When females died they were frozen for later dissection to determine ovarian and mating status. Larvae were reared individually in glass tubes with gauze lids or communally (up to 20 individuals) in a plastic container (16 × 28 × 7 cm) with a gauze lid. A small stem of *E. spurgulinum* bearing buds and flowers with the cut end in a small ball of moist cotton wool was provided for each larva in glass tubes and replaced every other day. Multiple stems with buds and flowers were provided to larvae in the plastic container and replaced after 3-4 days. Larvae were reared at 25-30°C under natural day lengths from July 17-August 15. Individually reared eggs and larvae were used to determine egg and instar sizes (using a microscope eyepiece graticule) and durations. Observations were made of larvae feeding, molting and pupating. High quality images were taken of eggs, all instars and pupae using a Canon digital SLR camera (EOS 1DS Mark II) mounted on a tripod. A Canon MP-E 65mm 1X – 5X macro lens was used together with a Macro Twin Lite MT – 24 EX flash lighting system.

Adult biology. Field observations on flight, sex ratio, nectaring, roosting, mating and oviposition behavior of *P. leona* adults were made during walks (12-15 hrs) through multiple patches of *E. spurgulinum* during each of six weekly visits to the Antelope Desert population from June 21-July 29 2011.

RESULTS

Observations during rearing in captivity

Oviposition. Oviposition by *P. leona* in captivity was poor. A total of only 12 eggs was obtained, 7 of these within 3 days from females obtained on July 1 and held individually with cut *E. spurgulinum* at flower bud stage. Three eggs were laid on potted *E. spurgulinum* by a group of 5 females held at 25-39°C under fluorescent lighting. The majority of females did not lay eggs, despite having well developed ovaries, as indicated by dissection of individuals that died. A mean of 39.3 ± 12.9 (SE) mature eggs were present in ovaries of six females that died during July 11-12,

compared to 12.6 ± 2.1 in six females that died during July 24-26 ($P = 0.015$, Mann-Whitney rank sum test, SigmaStat V 3.0). Eggs were mostly placed on or at the base of flower buds (Figs. 1-2). Two eggs were placed on a stem ~5 cm distant from buds.

Egg. The egg measures 0.48 ± 0.01 mm ($n = 12$) in width and is pale whitish-yellow (Fig. 1). The micropyle is relatively wide and contained within a slight depression. The surface is covered with a network of obscure ridges. Hatching occurs after 4-5 days at 25-27°C, taking ~1 hour with the larva eating a jagged hole through the top of the egg shell, exiting without consuming it (Fig. 2).

First instar. After hatching the first instar measures 1.0 ± 0.2 (SE) mm ($n = 10$). It is dull creamy-yellow, clothed with moderate length white setae arising from dark spots. The head is shiny brown (Fig. 2). After 24-36 hours of feeding, a green tinge develops. Light orange-red markings appear after 2 days forming a dorsal stripe and two indistinct lateral stripes. There is also a sub-spiracular pale stripe. Prior to molting the larva is suffused with increased reddish pigmentation and measures 2.0 ± 0.2 mm in length ($n = 8$) (Fig. 1).

Second instar. After molting to second instar the larva measures 2.0 ± 0.2 mm in length ($n = 8$) and is reddish with indistinct white markings which form two stripes laterally, the lower one better defined than the upper one. A darker red mid-dorsal stripe is present and the head is shiny black in this and all subsequent instars. Sparse, short white setae cover the body and the larva increases in length to 4.0 ± 0.1 mm ($n = 8$) (Fig. 1).

Third instar. The early third instar measures 4.0 ± 0.1 mm ($n = 8$) and is bright red with distinct white blotches dorsally and a prominent, wide sub-spiracular white stripe. The mid dorsal dark red stripe is broken into prominent spots, one per segment. The white setae are shorter and denser and the larva increases in length to 6.0 ± 2.0 mm ($n = 8$) (Figs. 1-2).

Fourth instar. The final instar develops from 6.0 ± 2.0 mm ($n = 8$) to 10.0 ± 3.0 mm ($n = 10$) and is mostly white with vivid blood-red markings, resulting from an interrupted mid-dorsal stripe and two lateral stripes either side of the sub-spiracular white stripe. Short white setae densely cover the body. Ventrally, the larva is red with yellow claspers and black true legs (Fig. 1).

Pre-pupa. When the larva reaches 7.0-10.0 mm in length, the dorsal white markings become suffused with red and it shrinks to 5.0-7.0 mm. After ~24 hours the larva becomes a sessile and uniformly yellow colored pre-pupa (Fig. 1). A single strand of silk may girdle the pre-pupa but this was seen in only 4 of 12 pupae examined.

Pupa. The pupa is uniformly orange-brown with darker wing cases and an indistinct mid-dorsal stripe on the abdomen. The spiracles are dark brown (Fig. 1). Pupa length ranges from 4.0-7.0 mm (mean 5.5 \pm 0.25 (SE) (n = 11)). Pupation occurred mostly on the ground (n = 9) but three pupae were formed on host plant stems.

Larval development and behavior. Development from egg hatch to pre-pupa was rapid at 25-27°C occupying 10-12 days. Larval development may be even faster in the field as judged by the relative proportions of instars in samples of larvae obtained on July 24 and 29. During these 5 days the larval population changed from early instar (1 & 2) dominated (73%, n = 26) to late instar (3 & 4) dominated (92%, n = 25). All instars fed only on the unopened flower buds and flowers of *E. spargulinum*. The petals were eaten together with other flower parts including perhaps pollen (Fig. 2). If flowers were not available all early instar (1-3) larvae died. Two final instar larvae attempted early pupation while only measuring 6-7mm in length. Both produced deformed, non-viable pupae. The pre-pupal stage is relatively prolonged occupying 5-6 days. This is unusual in northwest US lycaenids (James and Nunnallee 2011). In captivity, most prepupae were formed at the bottom of the cage and pre-pupal wandering was not observed. The pupal period lasts for at least 10-11 months but some pupae may remain dormant for more than a year. Dave McCorkle (pers. comm.) reports that some *P. leona* pupae remain dormant for 2 years in captivity before eclosing. From approximately 45-50 larvae reared all were the red color form described above. Matheson *et al.* (2010) reported the rare occurrence of a green larval form. Field observations in 2011 suggest the incidence of green form larvae to be ~ 1 in 100 (Gary Pearson, pers. comm.).

Observations on adult biology

The flight period occupied 6-7 weeks, from about June 18 to July 31. Males emerged a few days before females, producing an early male-skewed sex ratio. From early July until the end of the flight period the sex ratio was relatively balanced (Table 1). Spontaneous flight behavior did not generally occur until mid-late morning when air temperatures were > 21°C. Cloudy and/or windy conditions inhibited flight. Flight is low (usually < 0.3 m above the ground) and meandering with little or no straight line flight. Males search for females among patches of host plant and mating occurs soon after female eclosion. During late June-early July mating pairs

were frequently (5-10 pairs/day) seen resting on *E. spargulinum* and other low-growing plants, usually during late afternoon. Oviposition occurred from late June to the end of the flight period, peaking in early July (Table 1). Eggs were laid (mostly singly but occasionally two) only on budding *E. spargulinum* (25 observations). Nectaring was observed on many low growing flowering plants but was most common on *E. spargulinum*, *Gayophytum diffusum* Torr. & A. Gray (Onagraceae), *Attenaria rosea* Greene (Asteraceae), *Plagiobothrys hispidus* A. Gray (Boraginaceae), *Hemizonella minima* A. Gray (Asteraceae) and later in the flight period *Eriogonum umbellatum* Torr. (Polygonaceae). Males were observed to visit up to 67 flowers of *H. minima* in 12 minutes. Both sexes spent much time roosting or resting on dead twigs, dried grass blades and seed heads just above the ground. During morning hours, resting individuals oriented themselves so that ventral wing surfaces face the sun.

DISCUSSION

This study provides the first detailed observations on the life history and adult biology of *P. leona*. Rearing was relatively easy, however, obtaining oviposition from females was difficult. Best success was obtained with separately-caged individuals supplied on July 1 with budding *E. spargulinum*. Field observations indicated that *P. leona* oviposits only on budding *E. spargulinum* and the confinement of females with flowering rather than budding host plants on July 7 and 25 may have suppressed oviposition. Timing of larval feeding is critical, with flowers of *E. spargulinum* providing the apparent sole source of nourishment. Larvae of *Philotiella speciosa* also feed only on flowers when using the buckwheat, *Eriogonum reniforme* Torr. and Frem. (closely related to *E. spargulinum*) (Scott, 1986). Flower and seed feeding on *Eriogonum* spp. is also widespread in *Euphilotes* spp. (Pratt, 1994; Peterson, 1997). The immature stages of *P. leona* show typical lycaenid characters particularly those found in the related genus *Euphilotes*. The egg appears to be smooth but has fine undulating, irregular surface ridges. The micropyle-containing depression on the top of the egg appears to be unique among lycaenid species in the Pacific Northwest (James and Nunnallee 2011). The vibrant and contrasting red and white marked third and fourth instar larvae are cryptic on the reddish host plant and bear some resemblance to the last instar larvae of *Euphilotes columbiae* (Mattoni) and *Euphilotes enoptes* (Boisduval) (James and Nunnallee 2011). The apparent fourth instars of *P. leona* shown

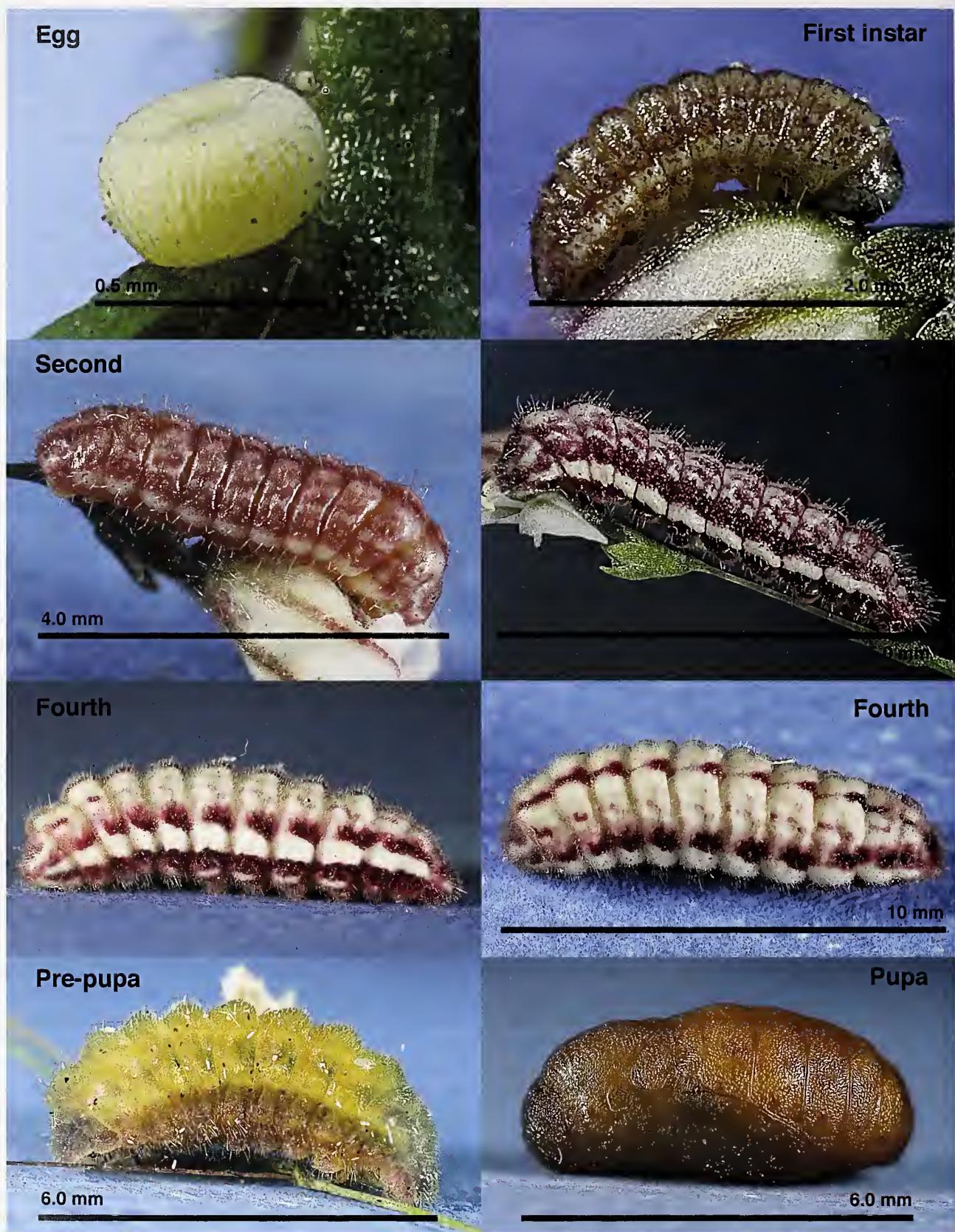


Figure 1. Life cycle stages of *P. leona*.

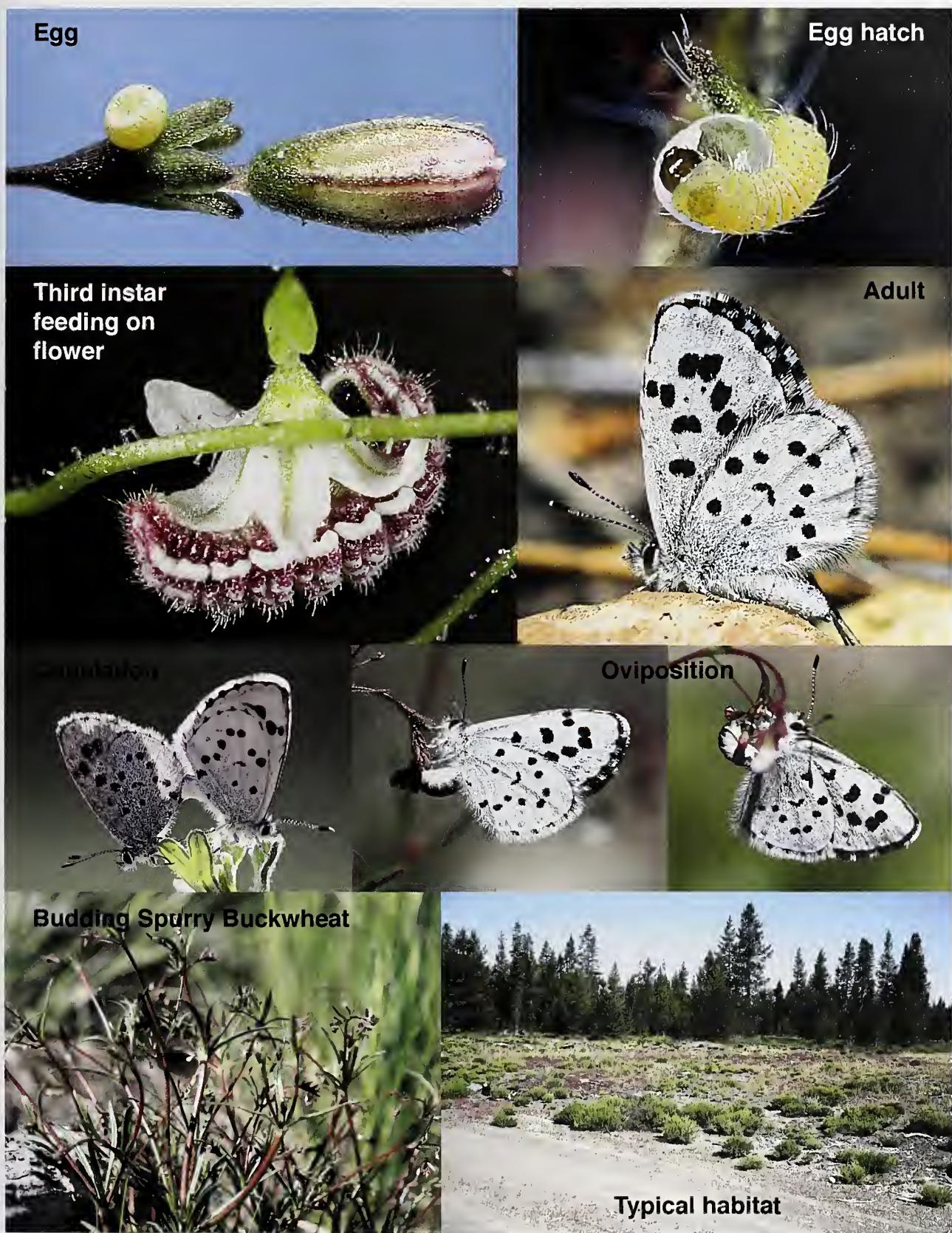


Figure 2. Aspects of the biology and ecology of *P. leona*

Table 1. Sex ratio (%) of *P. leona* adults captured or observed during June 21-July 28 2011. Bottom line shows number of oviposition events observed at each visit.

Sex	June 21-22	June 30-July 1	July 7-9	July 15-16	July 22-23	July 28-29
Male (%)	100	64.7	58.1	41.3	69.4	40
Female (%)	0	35.3	41.9	58.7	30.6	60
Total (n)	33	184	148	63	36	5
Oviposition events (n)	0	3	15	9	0	0

in Ross (2008, 2009) and Matheson *et al.* (2010) are identical to ours. The larva shown in Miller & Hammond (2007) is greener but has a similar mid-dorsal dark red stripe. The apparent low incidence of green form larvae is unsurprising given their conspicuousness on the predominantly red host plant. Color polymorphism is common in Lycaenidae larvae and often related to host plant color variation (Ballmer & Pratt, 1988; James & Nunnallee, 2011). Mature larvae of *P. speciosa* may be similar but in Allen *et al.* (2005) the larva of *P. speciosa* is dull red and lacks strongly contrasting white markings. Ballmer & Pratt (1988) described the ground color of *P. speciosa* larvae as 'green or yellowish'.

The observations presented here provide the basis for further laboratory and field studies on the biology and ecology of *P. leona*. In particular information is needed on the impact of weather and natural enemies on survival of immature stages and adults. The likelihood of extended pupal dormancy also needs investigating. Prolonged pupal diapause has been reported in some *Euphilotes* spp. (Austin *et al.*, 2008; Pratt & Ballmer, 1987) and in *Glaucomysche piasus* (Boisduval) (James & Nunnallee, 2011). It has also been reported for the closely related *P. speciosa* (Monroe & Monroe, 2004; Allen *et al.*, 2005) and appears to occur frequently in desert butterflies generally (Sands & New, 2008; James & Nunnallee, 2011). Critically, we need data on the population dynamics of *P. leona* over several years together with information on environmental variables, to assess whether the population is in decline, increasing or stable. A comprehensive set of data on the biology and ecology of *P. leona* will enable the development of land management strategies that permit the best chance of survival for this charismatic but severely restricted species.

ACKNOWLEDGEMENTS

I thank the Oregon Zoo Future for Wildlife Grants Program for providing partial funding for this research and Dave McCorkle for introducing me to Leona's Little Blue. Tanya, Jasmine and

Rhiannon James provided excellent help with field observations and cultivation of *E. spurgulinum*. I also thank Lorraine Seymour, Terri Knoke and Tim Yager for additional laboratory field observations and help in obtaining livestock.

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Uncus shaped akin to elephant tusks defines a new genus for two very different-in-appearance Neotropical skippers (Hesperiidae: Pyrginae)

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Abstract. Analyses of male genitalia, other aspects of adult, larval and pupal morphology, and DNA COI barcode sequences suggest that *Potamanaxas unifasciata* (C. Felder & R. Felder, 1867) does not belong to *Potamanaxas* Lindsey, 1925 and not even to the Erynnini tribe, but instead is more closely related to *Milanion* Godman & Salvin, 1895 and *Atarnes* Godman & Salvin, 1897, (Achlyodini). Unexpected and striking similarities are revealed in the male genitalia of *P. unifasciata* and *Atarnes hierax* (Hoppfer, 1874). Their genitalia are so similar and distinct from the others that one might casually mistake them for the same species. Capturing this uniqueness, a new genus *Eburuncus* is erected to include: *E. unifasciata*, new combination (type species) and *E. hierax*, new combination.

Key words: phylogenetic classification, monophyletic taxa, immature stages, DNA barcodes, *Atarnes sallaei*, Central America, Peru.

INTRODUCTION

Comprehensive work by Evans (e.g. Evans, 1937; 1952; 1953) still remains the primary source of information about Hesperiidae worldwide. Evans' vision as an evolutionary biologist has added to this work's influence, and the backbone of his taxonomic arrangements reflected in identification keys has stood the test of time. In particular, the order in which the species are arranged in the keys frequently approximates our present understanding of their phylogeny. Recent revolutionary studies that shaped our views of Hesperiidae phylogeny re-aligned some of the Evans groups and introduced a molecular basis for higher classification (Warren *et al.*, 2008; Warren *et al.*, 2009). However, a more detailed second look at specific taxa reveals and rectifies numerous classification mistakes at the genus level, as masterfully done by Burns in a series of papers (e.g.

1982-1999). Most of Burns' work derives from careful analysis of genitalia, recently assisted by morphology of immature stages and molecular evidence (e.g. Burns & Janzen, 2005; Burns *et al.*, 2009; 2010). Bit by bit, the classification of Hesperiidae is being adjusted to reflect their phylogeny.

Another interesting case, which caused a long-lasting confusion with regard to classification, is discussed here. A curious statement can be found in Evans (1953: 138) about the genus *Potamanaxas*: "Superficially a compact genus: structurally *unifasciata* is abnormal in respect of the secondary sexual characters." To contrast it with all other 11 species of the genus, *P. unifasciata* is placed first in Evans' *Potamanaxas* key, with all these "abnormal" characters listed, and he states: "gnathos absent." A few pages above (p. 131), the "gnathos absent" statement also appears for *Atarnes hierax*, but not for its congener *A. sallaei* (C. Felder & R. Felder, 1867). *A. hierax* is characterized by: "arms of uncus very long and slender." The genitalic sketches for *A. hierax* and *P. unifasciata* (plate 42, E.45.2 and E.49.1) look very similar to each other and very different from those of their congeners (e.g. E.45.1. and E.49.2 for comparison); being similar to the extent that allowing for imprecision in drawing, they may be taken to depict the same species. Did Evans mix up the genitalia and illustrate the same species under different names?

A combination of evidence from genitalia,

Received: 19 September 2012

Accepted: 13 November 2012

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morphology of immature stages, and COI mtDNA sequences strongly argues that *P. unifasciata* and *A. hierax* have been misclassified and attributed to genera they do not belong in. Moreover, despite a highly distinct superficial appearance, they are likely to be each others' closest known relatives. For these reasons, a new genus *Eburuncus* is erected for them.

MATERIALS AND METHODS

Specimens used in this study were from the following collections: National Museum of Natural History, Smithsonian Institution, Washington, DC, USA (USNM); Museum für Naturkunde, Berlin, Germany (ZMHB); Natural History Museum, London, UK (BMNH); and Texas A&M University Insect Collection, College Station, TX, USA (TAMU). Standard entomological techniques were used for dissection (Robbins, 1991), i.e. the adult abdomen being soaked for 24 hours in 10% KOH at room temperature, dissected and subsequently stored in a small glycerol vial pinned under the specimen. Genitalia and wing venation terminology follow Klots (1970) and Comstock (1918), respectively. Most photographs were taken using a Nikon D200 camera; for specimens through a 105mm f/2.8G AF-S VR Micro-Nikkor lens, and for genitalia through a transillumination microscope. DNA sequences reported in Janzen *et al.* (2011) were downloaded from GenBank <http://www.ncbi.nlm.nih.gov>, aligned by hand since insertions or deletions were absent, and analyzed using the Phylogeny.fr server at <http://www.phylogeny.fr> with default parameters (Dereeper *et al.* 2008).

History of *P. unifasciata* and *A. hierax* taxonomy

Leucochitonea unifasciata C. Felder & R. Felder, 1867 was named from "Nova Granada: Bogota", today's Colombia, from an unstated number of male specimens. A syntype is in BMNH (examined, photographed, dorsal side available in Warren *et al.*, 2012). Listed once as *Entheus unifasciata* by Kirby (1871) and *Pythonides unifasciata* by Plötz (1884), it was finally and stably placed in the newly described genus *Potamanax* (Watson, 1893; type species *Leucochitonea flavofasciata* Hewitson, 1870). Since this genus name was already occupied by Pilsbry, [1893] (in Mollusca) for about 4 months at the time of description, *Potamanax* was suggested by Lindsey (1925) to replace Watson's junior homonym. With the odd exception of Lewis (1973) and Moreno *et al.* (1998) who placed it in *Carrhenes* Godman & Salvin, 1895, *unifasciata* has always been listed in *Potamanax/Potamanaxas* or its various misspellings since Watson (1893; Mielke, 2005).

Milanion marica Godman & Salvin, 1895 was named from Nicaragua: Chontales in the newly described genus *Milanion* (type species *Papilio hemes* Cramer, 1777). The holotype (by monotypy) is in BMNH (examined, photographed, dorsal side available in Warren *et al.*, 2012). Godman and Salvin (1895) expressed some doubt about the generic assignment of *marica*, writing: "It bears some resemblance to *Potamanax*." Evans (1953) synonymized *marica* with *P. unifasciata*, which does not conflict with the evidence available to me.

Pythonides hierax Hopffer, 1874 was named from Peru: Chanchamayo. A series of 2 likely and 2 possible syntypes is in ZMHB (examined, photographed, available in Warren *et al.*, 2012). Plötz (1884) named this species *P. servatius*, a name which is therefore an objective junior synonym. Mabille (1903) transferred it (as *servatius*) to the genus *Atarnes* Godman & Salvin, 1897 (type, and only included species *Leucochitonea sallaei* C. Felder & R. Felder, 1867), where it has resided since.

RESULTS

Generic placement of *P. unifasciata* and *A. hierax*

Adults: As stated above, *unifasciata* should not be included into *Potamanaxas*. All *Potamanaxas* species possess in their male genitalia a gnathos, uncus arms that are shorter than or equal to the tegumen in length (Figs. 4gk), and brush organs at the bases of valvae. In addition, they are all characterized by the absence of secondary sexual characters in males and the absence of forewing subapical hyaline spots (Evans, 1953) (Figs. 1ij, 2ef). "*P.*" *unifasciata* lacks a gnathos and its uncus arms are at least 1.5 times longer than the tegumen (Figs. 4ab); its males possess several notable secondary sexual characters, such as a thoracic pouch, hair tuft on the hind tibia (Fig. 3a) and a vestigial costal fold; and the forewing of both sexes has distinct subapical hyaline spots (usually all 5 of them are developed; Figs. 1ab, 2ab). These differences were noted in Evans (1953), who contrasted *unifasciata* with all the rest of *Potamanaxas* species in his key. In addition, the light discal bands on the forewing of *Potamanaxas* do not show hyalinity (Figs. 1ij, 2ef). In contrast, *unifasciata* (Figs. 1ab, 2ab), like *Milanion* spp. (Figs. 1ef, 2c) and some other Achlyodini Burmeister, 1878 (Figs. 1cdgh, 2d), possesses hyaline areas in the discal band. The main similarity between *unifasciata* and some *Potamanaxas* species is the general look of the wing patterns: dark wings with white discal bands (Figs. 1abij, 2abef).

Larvae: Analysis of photographs of immature stages

corroborates that *unifasciata* is not a *Potamanaxas* (Fig. 5). As with many other Erynnini Brues & Melander, 1932 (photographs in on-line databases by Janzen & Hallwachs, 2012 and Warren *et al.*, 2012), later instar caterpillars of *Potamanaxas* (Fig. 5f) and the closely related *Mylon* Godman & Salvin, 1894 (Fig. 5g) possess a heart-shaped, trapezoid or almost triangular head, which widens dorsally as seen in anterior view. In photographed caterpillars representative of other Pyrginae Burmeister, 1878 tribes, especially in Achlyodini (apparently misspelled as Achlyodidini in Warren *et al.*, 2008; 2009), the head is more rounded, and is not significantly swollen dorsally in most cases (Figs. 5cd; Janzen & Hallwachs, 2012; Warren *et al.*, 2012). Although I could not find a photograph of a *unifasciata* caterpillar, an image of a pupa with larval skin still attached clearly shows a rounded head (Fig. 5a), very similar to that observed in *Milanion marciana* Godman & Salvin, 1895 (Fig. 5c). In addition, caterpillars of *unifasciata*, *M. marciana* and *Atarnes sallaei* all use Annonaceae as foodplants, while three *Potamanaxas* species reared from Guanacaste (NW Costa Rica) utilize Ericaceae (Janzen & Hallwachs, 2012). The immature stages of *A. hierax* have not been recorded.

Pupae: Pupal characters are even more revealing. Out of 18 genera placed in Erynnini by Warren *et al.* (2008; 2009), photographs of pupae are available for at least one species from 11 genera (Janzen & Hallwachs 2012; Warren *et al.*, 2012). All of these Erynnini taxa are characterized by a broad and stout pupa with short abdomen and a prominently developed pair of black spiracles on the thorax ('counterfeit eyes' per Janzen *et al.*, 2010). Erynnini pupae are almost always green, shiny and without bloom (Fig. 5h); however, after diapause, discolored caterpillars may produce whitish and brownish pupae as the one shown for *Erynnis persius fredericki* H. Freeman, 1943 (Fig. 5i). The pupal head capsule is rounded between the eyes. Certain genera of smaller Achlyodini skippers have more gracile, slender pupae with longer abdomens and smaller thoracic spiracles concolorous with the rest of the body, which is mostly whitish, yellowish or brown, sometimes covered in white bloom (Fig. 5e; Janzen & Hallwachs, 2012; Warren *et al.*, 2012). These Achlyodini display an anterior protuberance of the head capsule, sometimes consisting of a sharp horn-like point, with prominent concave surfaces formed between it and the eyes (pointed to by an arrow on Fig. 5e). The "P." *unifasciata* pupa (Fig. 5a) possesses these Achlyodini characters as listed above, rather than Erynnini characters.

DNA barcodes: The 654-nucleotide mitochondrial DNA sequence of the C-terminal region of the

cytochrome c oxidase subunit 1 (COI) gene, dubbed "barcode", offers further support for the placement of *unifasciata* among Achlyodini instead of in *Potamanaxas*, and not even among Erynnini. Six taxa with barcode sequences reported by Janzen *et al.* (2011) were chosen for analysis (Fig. 6). "P." *unifasciata* and *P. cf. hirta*, which is somewhat similar to *unifasciata* in wing pattern, represent Evans' *Potamanaxas*. *P. cf. hirta* called "Potamanaxas Burns01" (Janzen & Hallwachs, 2012) is an undescribed species somewhat similar to *P. hirta* (Weeks, 1901), and it has its DNA barcode sequence available (Janzen *et al.*, 2011). *Mylon lassia* (Hewitson, 1868) was used to represent a sister genus to *Potamanaxas*. *M. marciana* and *A. sallaei* are representatives of Achlyodini with similar immature characteristics to "P." *unifasciata*. Finally, *Drephalys alcmon* (Cramer, 1780) is from a different subfamily (Eudaminae Mabille, 1877) than the other taxa (Pyrginae Burmeister, 1878) and was used as outgroup to root the tree. Although barcode sequences are typically too short for confident phylogenetic inference, sometimes statistically supported and consistent results can be obtained. For instance, all four different phylogenetic methods offered at Phylogeny.fr web server (BioNJ, PhyML, MrBayes and TNT; Dereeper *et al.*, 2008) produced trees identical in topology, and statistical support for all internal nodes was close to 1. The trees according to the first and the last method mentioned above are shown in Fig. 6. One would expect the two *Potamanaxas* species to be sisters. However, that was not the case. *Mylon* and *P. cf. hirta* came out as sister taxa, but *unifasciata* grouped with *Milanion* and *Atarnes* on the other side from the root. Careful inspection of the multiple sequence alignment revealed the reasons (Fig. 6). At least 30 positions (red, magenta and green) voted against sister relationship between the two *Potamanaxas* species by supporting their grouping with other taxa, and only one position (blue) supported it. Furthermore, an analysis of evolutionary distances between sequences in terms of numbers (or %) of different nucleotides leads to the same conclusion. Distances were smallest between *unifasciata* and *Milanion* (6.4%) on the one hand, and between *P. cf. hirta* and *Mylon* (8.9%) on the other, suggesting these pairings of taxa to be sisters within the taxon sample under study. 6.4% is a very close distance indicative of tribal, and possibly even congeneric, relationship. Thus, DNA analysis aligns "P." *unifasciata* with *Milanion*, which agrees with Godman & Salvin's (1895) placement of its synonym *marica* in *Milanion*, and is inconsistent with a *Potamanaxas* that includes *unifasciata* being monophyletic. In summary, all lines of evidence, from



morphological to molecular, argue that *unifasciata* is not a *Potamanaxas*. DNA sequences of *A. hierax* have not been reported.

Finding a new genus for *unifasciata*: Among the genera *unifasciata* has been formerly placed in, none is suitable. The type species *levubu* Wallengren, 1857 of the original genus *Leucochitonea* belongs to Tagiadini Mabille, 1878, an exclusively Old World tribe (Warren, 2009), characterized by anal wool in females (Evans, 1937; Warren *et al.*, 2009). In addition, all three (exclusively African) species currently in *Leucochitonea* have a very short uncus (Evans, 1937). *Entheus* Hübner, [1819] is in a different subfamily (Eudaminae) and is characterized by very different palpi with the third segment being stout and spatulate, positioned close to the outer edge of the second segment (Evans, 1952). *Pythonides* Hübner, [1819] also differs in palpi: the 3rd segment is shorter than the 2nd, and in addition its uncus is undivided (Evans, 1953). *Carrhenes* Godman & Salvini, 1895 is characterized by a short uncus, developed gnathos, rounded wings with variegated pattern and a strongly developed costal fold in males. Finally, DNA barcodes are available for representatives of all these genera (except *Leucochitonea*), and they do not root close to *unifasciata* (data not shown).

Interestingly, when Evans' (1953) key for genera in group "E" is used to identify *unifasciata* and 49a (p. 15; "H costa and dorsum sub-equal") is correctly chosen over 40b (p. 13; "H longer at costa than at dorsum"), it keys to *Carrhenes* ("F vein 12 long, ending over end cell") instead of *Potamanaxas* ("F vein short and vein 11 ends over end cell"), except that the costal fold in males is vestigial. However, if 40b is selected over 49a, then it keys to the choice between *Atarnes* (broad white forewing band, orange spot on the forewing) and *Milanion* (hyaline forewing spots, "no red or

yellow spots"). Many specimens of *unifasciata* exhibit a narrow area of orange scales along the basal edge of the forewing white discal band, more prominent in the posterior half (Figs. 1b, 2a). This area does not quite qualify as the "spot" of *Atarnes*, but in combination with the white forewing band it suggests the choice of *Atarnes* over *Milanion* in the Evans key. However, placing *unifasciata* in *Atarnes* causes problems with monophyly of this latter genus in DNA barcode data, since *unifasciata* is sister to *Milanion* and only their common ancestor is sister to *Atarnes* *sallei* (Fig. 6), a strongly supported hypothesis. Alternatively, it seems plausible to place *unifasciata* in *Milanion* instead, and simply change the Evans key.

However, further look into *Atarnes* reveals a more compelling option. Indeed, the two species in *Atarnes*, *sallei* and *hierax*, have very different genitalia (Fig. 4), as noted in Evans (1953). The *A. sallei* uncus is much shorter, the gnathos is present, its valva lacks a style, and the harpe is quite extended without a prominent tooth dorsad (Figs. 4hl). The *A. hierax* uncus is very long, about twice as long as the tegumen, the gnathos is absent, and the valva has a prominent style and a large tooth on a shorter harpe (Figs. 4cdei). Although both species possess a thoracic pouch (Figs. 3bc), it is somewhat different in shape, being wider in *A. sallei* (Fig. 3c). The *A. hierax* pouch is more slender (Fig. 3b), and similar to that of "*P.* *unifasciata*" (Fig. 3a). Even wing patterns, despite superficial resemblance, reveal curious differences. In *A. hierax*, white streaks are along the veins (Figs. 1cd), but in *A. sallei* they are mostly between the veins in the middle of each cell (Figs. 1gh). Moreover, the position of the orange forewing spot differs. In *A. hierax* this spot is bordering the basal boundary of the white discal band and is more diffuse and absent ventrally, but in *A. sallei* it is inside the brown basal area, is more defined, is

Figure 1 (Opposite page). Spread adults. The letter is placed between the images of the same specimen. Dorsal above or left, ventral below or right. 1cm scale is shown for each specimen between the two images. "F" indicates that a mirror image (i.e. left-right inverted) is shown. Unless indicated otherwise (c, d, g.), specimens are from Costa Rica, Area de Conservacion Guanacaste and are in USNM collection. a, b. *Eburuncus unifasciata*, a. ♂ Guanacaste Prov., Guacimos, 380m, collected on 21-VI-2006, eclosed on 07-VII-2006, foodplant *Annona rensoniana* (Annonaceae), voucher code 06-SRNP-21553; b. ♀ Guanacaste Prov., Sector Cacao, Sendero Arenales, 1080m, collected on 15-VIII-1994, eclosed on 19-VIII-1994, foodplant *Annona rensoniana* (Annonaceae), voucher code 94-SRNP-6414; c, d. *Eburuncus hierax*, c. likely syntype ♂ Peru: Chanchamayo, leg. Thamm, [ZMHB]; d. ♂ Peru, from Geo G. MacBean, genitalia NVG120922-36 [USNM], (genitalia on Fig. 4de, thoracic pouch on Fig. 3b); e, f. *Milanion marciana*, e. ♂ Alajuela Prov., Sector Rincon Rain Forest, Camino Rio Francia, 410m, collected on 28-IX-2004, eclosed on 18-X-2004, foodplant *Annona papilionella* (Annonaceae), voucher code 04-SRNP-42477; f. ♀ Alajuela Prov., Sector San Cristobal, Sendero Huerta, 527m, collected on 02-III-2007, eclosed on 31-III-2007, foodplant *Annona rensoniana* (Annonaceae), voucher code 07-SRNP-1128; g, h. *Atarnes sallei*, g. ♂ Mexico: Tamaulipas, Sierra Cucharas, nr. rock quarry, ex larva, foodplant *Annona globiflora* (Annonaceae), eclosed 28-I-1975, leg. Roy O. Kendall & C. A. Kendall [TAMU]; h. ♀ Guanacaste Prov., Sector Mundo Nuevo, Mamones, 365m, collected on 24-VIII-2006, eclosed on 19-IX-2006, foodplant *Annona rensoniana* (Annonaceae), voucher code 06-SRNP-57921; i, j. *Potamanaxas* cf. *hirta* (Burns01), i. ♂ Guanacaste Prov., Sector Pitilla, Sendero Memos, 740m, collected on 29-III-2007, eclosed on 18-IV-2007, foodplant *Cavendishia axillaris* (Ericaceae), voucher code 07-SRNP-31875; j. ♀ Guanacaste Prov., Sector Pitilla, Sendero Memos, 740m, collected on 16-IV-2011, eclosed on 13-V-2011, foodplant *Cavendishia axillaris* (Ericaceae), voucher code 11-SRNP-31012. Pinholes and some other imperfections have been digitally removed to emphasize all actual elements of the pattern, such as small white spots.



Figure 2. Live adults. a, b. *Eburuncus unifasciata*, dorsal and ventral views, Mexico: Veracruz, Ruiz Cortines, 5-VI-2008, Bill Bouton; c. *Milanion* sp., Panama: Darien, Cana Field Station, 07-I-2003, Will & Gill Carter; d. *Atarnes sallaei* Mexico: Tamaulipas, Los Troncones Canyon, 18-XI-04, Kim Davis & Mike Stangeland; e, f. *Potamanaxas* spp. cf. *hirta* or *thoria*, e. Ecuador: Pastaza Province, Palora, Santa Rosa, -1.43° -78.00°, 900m, 19-VIII-2011, Pierre Boyer; f. Colombia: Dept. Risaralda, Otun, Quimbaya Reserve above Pereira, 1800m, 13-IX-2010, Kim Garwood. Hyaline areas are visible as darker patches inside the white forewing bands in a-d. No hyalinity is seen in e, f. *P.* spp.

present on both wing surfaces and is separated from the white discal band by patches of brown scales. Due to these genitalic and wing pattern differences in the two *Atarnes* species, and the profound similarities of *A. hierax* genitalia with those of *P. unifasciata* (Fig. 4ab), it is most likely that the streaky orange-spotted patterns are convergent. "*P.*" *unifasciata* is a likely sister species of *A. hierax*, which would make *Atarnes* polyphyletic. Thus, to be consistent with all available data and to suggest a phylogenetic hypothesis best supported by existing evidence, either *A. hierax* together with *P. unifasciata* should be transferred to *Milanion*, or a new genus should be erected for these two taxa. Since all seven *Milanion* species are quite close to each other in wing patterns and genitalia (Evans, 1953), thus forming a tight cohesive group, and none of them possesses a long uncus, a style on the valva and lacks the gnathos (Fig. 4fj), it appears that a new genus hypothesis should be preferred, and this new genus is named here.

Eburuncus Grishin, new genus

(Figs. 1a-d, 2ab, 3ab, 4a-d,i, 5ab)

Type species: *Leucochitonea unifasciata* C. Felder & R. Felder, 1867

Diagnosis: Very long and slender uncus arms (more than 1.5x tegumen length) combined with the absence of a gnathos is the defining character and a synapomorphy for the genus (Figs. 4a-d,i). Other possible synapomorphies that are present in all

known species of this genus include the shape of the valva with a rectangular process, the harpe with a prominent single tooth on the dorsal surface, and a forewing pattern consisting of a white discal band partly hyaline distally in discal cell and cell Cu₁-Cu₂, frequently with areas of orange scales along its basal edge, more developed in the posterior part, and subapical hyaline spots (Figs. 1a-d, 2ab). A combination of these characters differentiates species of this new genus from related or similar taxa. Other characters are detailed in the description.

Description. **Forewing** Sc vein long, reaching the end of discal cell, background color brown, subapical hyaline spots, discal white band with hyaline areas in discal cell and cell Cu₁-Cu₂, frequently with a patch of orange scales along the basal edge, more expressed in the posterior half. **Hindwing** margin brown, base brown dorsally and lighter ventrally, white discal band or patch near costa. **Antenna** about half of costa length, bent from beginning of nudum of 14-16 segments, apiculus about equal in length to the rest of club. **Palpi** porrect with the 3rd, narrower segment in the middle of 2nd segment and as long as the 2nd segment. **Male secondary sex characters:** hind tibiae with a tuft of scales fitting in a thoracic pouch. Costal fold either absent or vestigial. **Male genitalia:** gnathos absent, arms of uncus very long and slender, close to twice the length of tegumen and about half of valval length, sacculus short, shorter than the style on the valva. **Variation:** one species with whitened veins, broader forewing discal white band and larger orange spot; the other species with 5 instead of 2 forewing apical hyaline spots, a hyaline spot at the base of M₃-Cu₁ forewing cell and a broad discal band on the hindwing from costa to tornus instead of a white patch not reaching the tornus.

Species included: *Leucochitonea unifasciata* C. Felder & R. Felder, 1867 with its junior subjective synonym *Milanion marica* Godman & Salvin, 1895; and *Pythonides hierax* Hopffer, 1874 with its junior objective synonym *P. servatius* Plötz, 1884.

Etymology: The name is a composite of two words "ebur" (ivory, ivory objects, and also elephant) and "uncus" (the terminal



Figure 3. Thoracic pouch and tibial tufts. a. *Eburuncus unifasciata* Panama: Cerro Campana, 1500', 9-X-1966, leg. G. B. Small, genitalia NVG120207-01 [USNM] (genitalia on Fig. 4ab); b. *Eburuncus hierax*, Peru, from Geo G. MacBean, genitalia NVG120922-36 [USNM], (specimen on Fig. 1d, genitalia on Fig. 4de); c. *Atarnes sallaei* Costa Rica: Guanacaste Prov., Area de Conservacion Guanacaste, Sector Mundo Nuevo, Porton Rivas, 570m, collected on 07-VIII-2007 as penultimate instar, eclosed on 26-VIII-2007, foodplant *Annona pruinosa* (Annonaceae), voucher code 07-SRNP-58936, genitalia NVG120922-28 (Fig. 4h) [USNM], mirror image (=left-right inverted).

hook-like structure in the male genitalia). It points to the defining character of the genus: a very long uncus with arms shaped akin to elephant tusks. Although not as provocative as *Cornuphallus* (translation: “horny dick”, jokingly coined by George T. Austin), *Eburuncus* (translation: “tuskyhook”) continues the interesting tradition suggested by G. T. Austin of naming genera by peculiar features of male genitalia. The name is a masculine noun in the nominative singular.

DISCUSSION

Male genitalia remain the primary guiding bedrock for Hesperiidae classification as they are diverse and rarely undergo convergence. The likely convergent streaky patterns of *A. sallaei* and *E. hierax* remind us that to achieve classification better reflecting evolutionary relationships, every hesperiid genus should be scrutinized and male genitalia carefully examined. To detect convergent patterns, close comparison is in order. Superficial streaky appearance is intrinsically different in these two species. *E. hierax* has white veins, but *A. sallaei* has white rays developed mostly between the veins in the middle of each cell. The orange spot is a part of the edge between the brown base and white discal band in *E. hierax* (as it is in *E. unifasciata*), but is a semi-square area inside the brown base in *A. sallaei*.

While it is not possible to confidently predict what yet undiscovered species, if any, would be placed in this new genus and thus what characters, especially wing patterns, they would possess, the description of *Eburuncus gen. nov.* summarizes those characters that are likely to be synapomorphic, including wing

patterns. These characters, in accordance with ICBN Code (1999) Article 13.1.1 and Recommendation 13A, are common to both species in the genus and differentiate them from all other described species. However, for newly discovered species attribution to the genus should be based on phylogenetic considerations rather than a blind application of all the characters listed in the description, as it is possible that some of these characters may not portray some of the species yet to be discovered.

A genus, being a primary taxonomic group between the family and species ranks, should be defined with care. Both over- and undersplitting hinders communication between researchers. When a new genus is proposed, a primary concern should be whether an unnecessary synonym might be created. Standards in defining a genus may vary between taxa and the history of generic usage for a particular group should be taken into account. A new genus should be most consistent with genera traditionally in use for the closest taxa. Current evidence suggests that *Eburuncus gen. nov.* and *Milanion* are sisters. Thus, in principle, *E. unifasciata* and *E. hierax* could be treated under *Milanion* to create a larger and more diverse genus. However the hiatus between *Eburuncus gen. nov.* and *Milanion*, taking into account the similarity in genitalia within each genus, is quite large. Wing patterns also readily distinguish them. Both *Eburuncus gen. nov.* species have a complete white discal band on the forewing starting from the costa and frequently an area of orange scales. In *Milanion*, the band is incomplete and does not reach the costa,

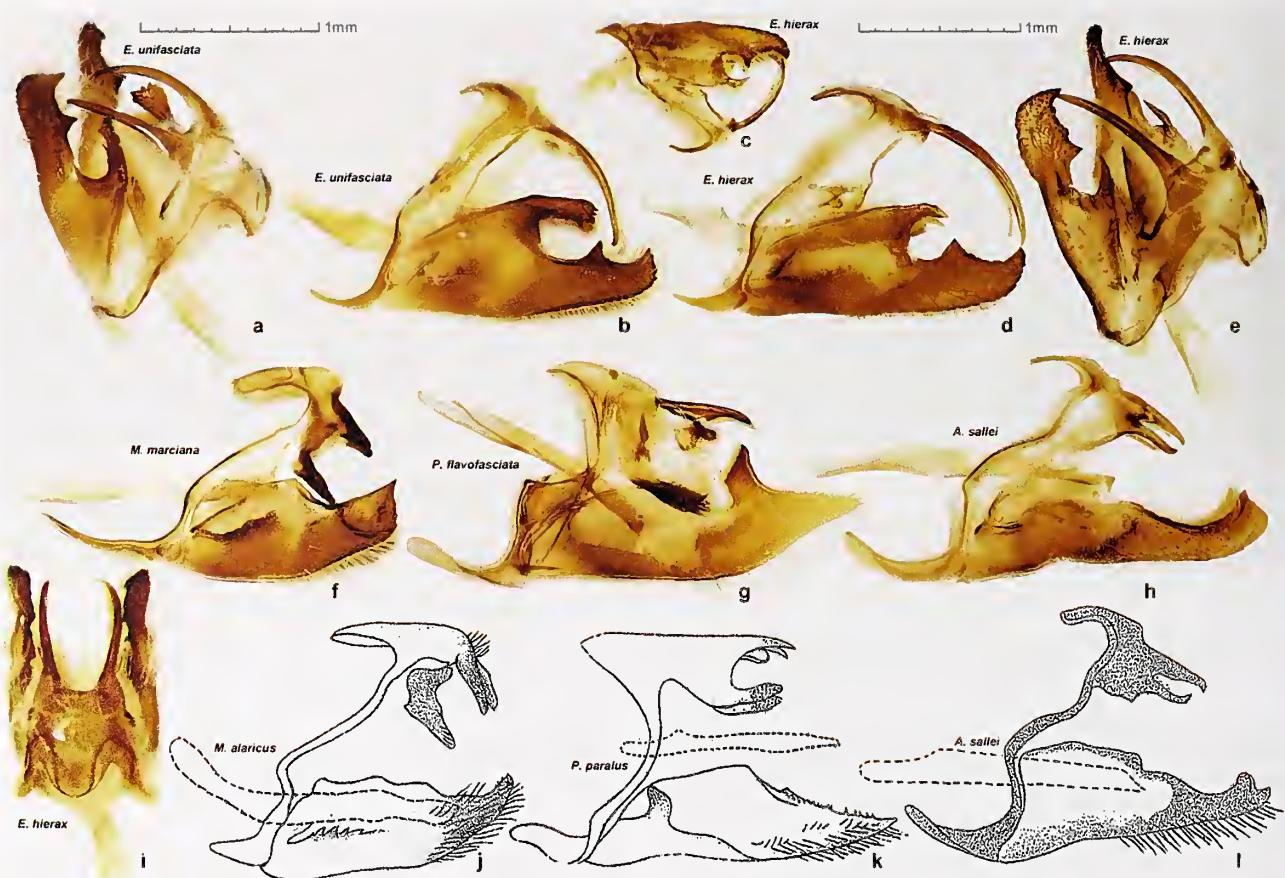


Figure 4. Male genitalia. a, b. *Eburuncus unifasciata* Panama: Cerro Campana, 1500', 9-X-1966, leg. G. B. Small, genitalia NVG120207-1 [USNM] (thoracic pouch on Fig. 3a); c, i. *Eburuncus hierax*, likely syntype, Peru: Chanchamayo, leg. Thamm, genitalia NVG120717-3 [ZMHB]; d, e. *Eburuncus hierax*, Peru, from Geo G. MacBean, genitalia NVG120922-36 [USNM], (specimen on Fig. 1d, thoracic pouch on Fig. 3b). f. *Milanion marciana* Costa Rica: Alajuela Prov., Area de Conservacion Guanacaste, Sector Rincon Rain Forest, Camino Rio Francia, 410m, collected on 28-IX-2004, eclosed on 18-X-2004, foodplant *Annona papilionella* (Annonaceae), voucher code 04-SRNP-42477, genitalia NVG120922-25 [USNM]; g. *Potamanaxas flavofasciata flavofasciata* (Hewitson, 1870), Peru: Amazonas, 4km W Abra Wawajin, 05° 18'S 78° 24'W, 750m, 24-IX-1999, leg. R. K. Robbins & G. Lamas, genitalia NVG120922-32 [USNM]; h. *Atarnes sallaei*, Costa Rica: Guanacaste Prov., Area de Conservacion Guanacaste, Sector Mundo Nuevo, Porton Rivas, 570m, collected on 07-VIII-2007 as penultimate instar, eclosed on 26-VIII-2007, foodplant *Annona pruinosa* (Annonaceae), voucher code 07-SRNP-58936, genitalia NVG120922-28 [USNM] (thoracic pouch on Fig. 3c); j. *Milanion alaricus* (Plötz, 1884), pl. 87, f. 1, identified by Evans (1953), not *M. hemes* or *M. leucaspis* (Mabille, 1878) as in the Godman & Salvini (1895) plate caption or text; k. *Potamanaxas paralus* (Godman & Salvini, 1895), original illustration of a syntype genitalia, Peru: Cosnipata Valley, leg. H. Whitely, pl. 86, f. 1; l. *Atarnes sallaei*, pl. 90, f. 16. Drawings e. and f. are from Godman & Salvini (1895) and g. is from Godman & Salvini (1897). Scale on the left refers to a, b, f, g, h. Scale on the right refers to d and e. Other images are not to scale.

and no orange scales are observed. Additionally, we see a trend in the recent literature to propose a finer generic structure for Hesperiidae, with smaller and more cohesive, frequently monotypic genera (Steinbauer, 1989; Austin & Warren, 2001; Austin, 1997; 2008). Therefore erection of *Eburuncus* gen. nov. is both biologically and historically justifiable.

Barcode sequences of mtDNA COI, although they are too short for confident phylogenetic inference, have been very helpful for the analysis of Hesperiidae diversity (Janzen *et al.*, 2011) and could be an excellent

source of phylogenetic hypotheses requiring further corroboration. Conversely, barcodes could be used as additional evidence along with morphological characters to support hypotheses suggested on the basis of other data. However, even in Hesperiidae, where the COI barcode typically correlates well with divergence of species, due to frequent cases of introgression (Zakharov *et al.*, 2009) it is not advisable to base phylogenetic conclusions entirely on a small sample of barcodes.

A COI DNA barcode distance of 6.4% agrees

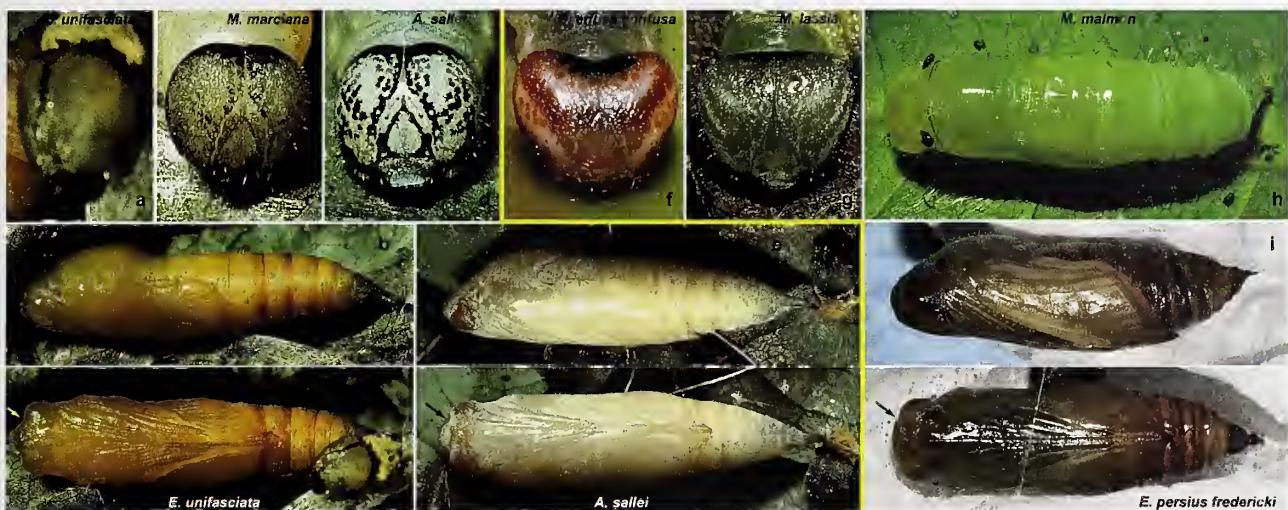


Figure 5. Immature stages. All specimens are from Costa Rica, Área de Conservación Guanacaste except i., which is from USA: Utah. a, b. *Eburuncus unifasciata*, Guanacaste Prov., Sector Cacao, Sendero Arenales, 1080m, collected on 10-II-1995, photographed on 25-II-1995, foodplant *Annona rensoniana* (Annonaceae), voucher code 95-SRNP-438, image codes DHJ21801 (a. and b. below) and DHJ21797 (b. above); c. *Milanion marciana*, Alajuela Prov., Sector Rincon Rain Forest, Camino Rio Francia, 410m, collected on 26-VI-2002, photographed on 09-VII-2002, foodplant *Annona papilionella* (Annonaceae), voucher code 02-SRNP-7566, image code DHJ67669; d, e. *Atarnes sallaei*, Guanacaste Prov., d. Sector Cacao, Sendero Maritza, 760m, collected on 19-II-1997, photographed on 16-III-1997, foodplant *Annona rensoniana* (Annonaceae), voucher code 97-SRNP-678, image code DHJ40302; e. Sector Santa Rosa, Las Mesas, 305m, collected on 09-XII-1992, photographed on 02-I-1993, foodplant *Annona purpurea* (Annonaceae), voucher code 92-SRNP-6105, image codes DHJ16973 (above) and DHJ16970 (below); f. *Potamanaxas effusa confusa* (Draudt, 1922), Alajuela Prov., Sector Brasilia, Piedrona, 340m, collected on 07-XI-2007, photographed on 19-XI-2007, foodplant *Satyria panurensis* (Ericaceae), voucher code 07-SRNP-65901, image code DHJ435325; g. *Mylon lassia*, Guanacaste Prov., Sector Del Oro, Quebrada Romero, 490m, collected on 21-VIII-2002, photographed on 07-IX-2002, foodplant *Cissampelos pareira* (Menispermaceae), voucher code 02-SRNP-28838, image code DHJ70894; h. *Mylon maimon* (Fabricius, 1775), Guanacaste Prov., Sector Santa Rosa, Vado Cuajiniquil, 5m, collected on 18-X-1993, photographed on 03-XI-1993, foodplant *Heteropteryx laurifolia* (Malpighiaceae), voucher code 93-SRNP-6934, image code DHJ26415; i. *Erynnis persius frederickii* H. Freeman, 1943 USA: Utah: Davis Co., Francis Peak, 12-(above) & 5-(below)-III-2007. a, c, d, f, g. Larval heads in anterior view, a. is a head capsule with skin still attached to pupa. b, e, h, i. Pupae. Except for h. (*M. maimon*) shown in dorsal view, others are shown in lateral and ventral views above and below, respectively; e. shows a mirror image (i.e. left-right inverted). Arrow points at an area between eye and anterior portion of the head capsule, which is concave in small Achlyodini skippers and mostly flat in Erynnini. All images are from the Janzen & Hallwachs database (2012) <http://janzen.sas.upenn.edu/caterpillars/database.lasso>, except i., which is by Nicky Davis.

well with the distances observed between species from very close genera in Eudaminae and Pyrginae (Janzen *et al.*, 2011). For instance, one of the smallest intergeneric distances is found between *Achalarus* and *Thessia* – only 3.5% between *A. toxus* (Plötz, 1882) and *T. jalapus* (Plötz, 1881). Comparable distances to *Eburuncus*-*Milanion* are seen between *Helioptetes* Billberg, 1820 and *Helioptygus* Herrera, 1957: 4.9% [*H. ericetorum* (Boisduval, 1852) and *H. domicella* (Erichson, [1849])]; *Pseudonascus* Austin, 2008 and *Nascus* Watson, 1896: 7.3% [*P. paulliniae* (Sepp, [1842]) and *N. solon* (Plötz, 1882)]; *Eantis* Boisduval, 1836 and *Achlyodes* Hübner, [1819]: 8.4% [*E. tamenund* (W. H. Edwards, 1871) and *A. pallida* (R. Felder, 1869)]; and *Salatis* Evans, 1952 and *Nicephellus* Austin, 2008: 8.9% [*S. canalis* (Skinner, 1920) and *N. nicephorus* (Hewitson, 1876)]. These distances are given here for

the purpose of comparison only, and application of a uniform COI % difference cutoff carries little value in defining a meaningful genus. For instance, the intrageneric distances between some species might be larger than some intergeneric distances, e.g. taking *Achlyodes* and *Eantis*, the distance between *A. pallida* and *A. busirus heros* Ehrmann, 1909 is about 9.5%, which exceeds the distance of 8.4% between *A. pallida* and *E. tamenund*. Each case needs to be analyzed individually and multiple factors considered, such as phylogenetic tree structure, internal branch lengths and statistical significance of nodes.

The example of *E. unifasciata* is quite similar to the example of *Helioptygus americanus* (Blanchard, 1852), which in dorsal wing pattern closely resembles *Pyrgus* Hübner, [1819] and was placed in it before genitalic similarities with *H. domicella* and *H. sublinea*

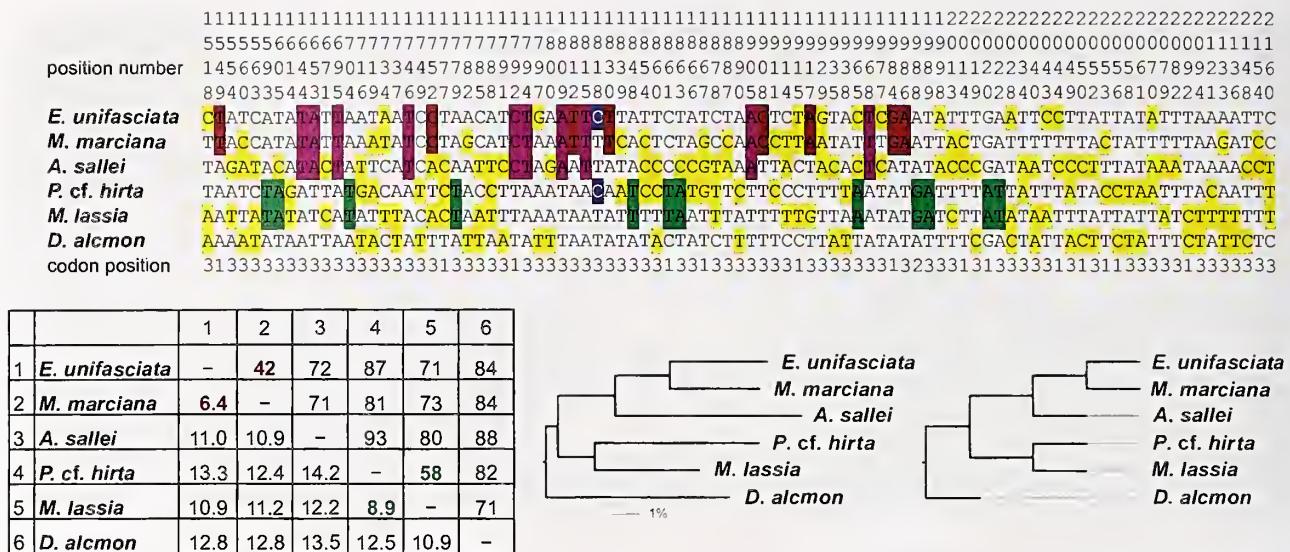


Figure 6. DNA-derived data. Multiple sequence alignment (top), distance matrix (bottom left), distance (bottom middle) and maximum parsimony (bottom right) trees are shown. Specimen voucher codes, Genbank accessions and NCBI GI numbers for each sequence are: *Eburuncus unifasciata*: 04-SRNP-364, DQ293104, 84099191; *Milanion marciana*: 04-SRNP-41660, DQ292619, 84098221; *Atarnes sallaei*: 06-SRNP-59199, JF760397, 332377953; *Potamanaxas cf. hirta*: 11-SRNP-31012, JQ526704, 374915892; *Mylon lassia*: 04-SRNP-47729, GU161659, 290548094; *Drephalys alcmon*: 00-SRNP-2692, JF752622, 333946838, respectively. *P. cf. hirta* (called "Potamanaxas Burns01") is a likely undescribed species with similarities to *P. hirta*. The aligned 654 positions of COI nucleotide sequences from 6 Hesperiidae species correspond to positions 1516-2169 in the *Drosophila yakuba* (Burka, 1954) mitochondrial genome, which is used as the numbering standard, because mitochondrial genomes have different lengths in different taxa. For brevity, only phylogenetically informative positions are shown in the alignment, i.e. invariant positions and positions with a nucleotide difference in a single sequence were removed. *D. yakuba* position numbers are shown above the alignment as columns (e.g., the first shown position is #1518). For each position, its place in a codon (1st, 2nd, or 3rd) is shown below the alignment ("codon position"). Majority of nucleotide changes in the 3rd position (marked as "3") do not cause a change in a protein encoded by the DNA, thus such changes are more common and are usually subject to more homoplasies. Nucleotides common to *E. unifasciata* and *M. marciana* in positions with nucleotides different from them in all other sequences are highlighted red. These 8 positions most strongly support grouping of these two taxa in a tree. Nucleotides common also to *A. sallaei* in addition to the above 2 species, but different from the rest in these positions are highlighted magenta. These 10 positions support grouping of these three taxa in a tree. Nucleotides common to *P. cf. hirta* and *M. lassia* in positions with nucleotides different from them in all other sequences are highlighted green. These 12 positions suggest a sister relationship between these two taxa. There is only a single position that supports *E. unifasciata* being sister to *P. cf. hirta*, (nucleotides in these taxa shown in white on blue), vs. 30 positions strongly supporting alternative trees. All other nucleotides except the most frequent one at each position are highlighted yellow. In the distance matrix, number of nucleotide differences and % of nucleotide differences between sequences are shown above and below diagonal, respectively. For BioNJ distance tree, scale bar corresponds to about 1% difference and all nodes received bootstrap support above 0.9. In TNT parsimony tree branches are not scaled. Topologies of the trees are identical to each other and are in agreement with red, magenta and green highlights in the alignment.

(Schaus, 1902) were brought to light (Herrera *et al.*, 1957; Austin & Warren, 2001). As a result of these studies, *H. domicella* and *H. sublinea* were extracted from the genus *Helioptetes* and united with *P. americanus* under the genus *Helioptyrus*. However, the remaining *Helioptetes* are quite diverse, so the possibility remains that the genus became paraphyletic as a result. It is not likely that *Milanion* is paraphyletic after exclusion of *Eburuncus* gen. nov., because all seven *Milanion* species are very similar to each other. The forewing lacking orange or red patches but with the white discal band broken into many spots may be synapomorphies for *Milanion* in comparison to the closely related genera

Eburuncus gen. nov., *Atarnes*, *Paramimus* Hübner, [1819], *Charidia* Mabille, 1903, and *Haemactis* Mabille, 1903 – all of which, at least in females, have orange or red patches on the forewing, and either an entire discal band or zero to two white spots, instead.

ACKNOWLEDGEMENTS

I am grateful to Robert K. Robbins, John M. Burns and Brian Harris (National Museum of Natural History, Smithsonian Institution, Washington DC), Wolfram Mey (Museum für Naturkunde, Berlin, Germany), David Lees and Blanca Huertas (Natural History Museum, London, UK), for granting access to the collections under their care and stimulating discussions;

Daniel H. Janzen and Winnie Hallwachs for the photographs of immature stages; Bill Bouton, Nicky Davis, Jean-Claude Petit, Pierre Boyer, Kim Garwood, Will and Gill Carter, Kim Davis and Mike Stangeland for the photographs of live individuals; Bernard Hermier for many enlightening discussions, numerous suggestions, and a most thorough and helpful review of the manuscript; Jonathan P. Pelham for insightful discussion of taxonomic issues and literature; Brian Bunker for critical reading of the manuscript and corrections; JRL editor Konrad Fiedler and an anonymous reviewer for helpful comments, modifications and edits. The work to obtain photographs and specimens from ACG has been supported by U.S. National Science Foundation grants BSR 9024770 and DEB 9306296, 9400829, 9705072, 0072730, 0515699, and grants from the Wege Foundation, International Conservation Fund of Canada, Jessie B. Cox Charitable Trust, Blue Moon Fund, Guanacaste Dry Forest Conservation Fund, Area de Conservación Guanacaste, and University of Pennsylvania to Daniel H. Janzen.

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A new species of *Neodactria* Landry, 1995 (Lepidoptera: Pyralidae, Crambinae) from Arizona, U.S.A.

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Abstract. So far only two species of *Neodactria* Landry have been found in the state of Arizona: *N. luteolellus* (Clemens, 1860) and *Neodactria cochisensis* Landry & Albu, sp. n. described and illustrated here from the Huachuca and Chiricahua Mountains of Cochise County. It differs from the other members of the genus most conspicuously by the curved and dorsally projecting costal process of the valva and in the female by the diminutive anterior apophyses and the regularly cylindrical corpus bursae.

Keywords: Lepidoptera, Pyraloidea, Pyralidae, Crambinae, *Neodactria*, new species, U.S.A., Arizona, Cochise County.

INTRODUCTION

In June-July 2011, the Sierra Vista region of south-eastern Arizona was immolated by the Monument Fire, one of the most devastating wildfires on record for that area. The second author (VA) visited the region between July 17 and 21, at the beginning of the monsoon season. By then the fire had been extinguished, but the charred tree remnants throughout the Huachuca Mountains remained as evidence of the devastation. Wildfires are a regular annual occurrence in south-eastern Arizona, especially before the monsoon season. Many of them are caused by lightning, but many also are started by human activities. These fires are an integral part of the natural, evolutionary forces that shape the local flora and fauna. In the last 100 years, human actions of prevention and containment of wildfires have resulted in an increase in the vegetation mass which in turn has fuelled ever more intense blazes. The year 2011 set a record in total acreage burned in Arizona,

over 1,000,000 acres by August 31st, according to the Arizona Geological Survey. The Monument Fire was relatively moderate in size (it burned around 30,500 acres over its 1 month life from June 12th to July 12th), but its raging over inhabited areas of the southern Huachuca Mountains, around the town of Sierra Vista, caused the greatest damage to man-made structures in the area.

The fire spread had been erratic and so, while some areas were totally consumed (e.g. Ash, Miller, and Carr Canyons), others were spared and sustained relatively little damage. This was the case of Ramsey Canyon. VA spent the time in a cabin on Ramsey Canyon Road (31.452444, -110.303491), at 1615 m altitude, close to the Ramsey Canyon Preserve. This is a mid-elevation locality in the Huachuca Mountains, one of South-eastern Arizona's "sky islands." These are mountain ranges rising abruptly from the surrounding lowlands of strikingly different habitats. They can ascend from the desert floor to pine and snow covered peaks. This proximity, yet isolation of the different mountains and their altitudinal zonation creates unique biogeographical conditions for floral and faunal endemism, relict populations, and vertical migrations. They present a strong analogy to a group of islands surrounded by channels of ocean. Among the Lepidoptera attracted to the light at the cabin were three small, unusual-looking crambines. Unable to identify them, VA consulted the first author (BL) as to their identity, and the new species status of this taxon was thus confirmed.

BL had collected himself one male in 1989 at Ash

Received: 21 November 2012

Accepted: 28 November 2012

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Figure 1. Holotype of *Neodactria cochisensis* sp. n.

Canyon, and he had associated it with a potentially conspecific female collected by J. Brown in 1986 at Turkey Creek in the Chiricahua Mountains. These specimens remained undescribed until now for lack of material.

Genus *Neodactria* Landry, 1995 contains only eight species according to the most up-to-date list available (Nuss *et al.*, 2012). Three of them were described during the last decade (Landry & Metzler, 2002; Landry & Brown, 2005).

MATERIAL AND METHODS

At the type locality VA collected three specimens, that became the holotype and two paratypes, with a 150 W mercury vapour lamp set next to a white sheet. The same method was used by BL and J.-F. Landry when they collected the other known male at Ash Canyon.

Terminology follows Landry (1995) except for the use of 'phallus', instead of 'aedeagus' as recommended by Kristensen (2003).

The following acronyms are used: MHNG for Muséum d'histoire naturelle de Genève, Geneva, Switzerland; UCB for Essig Museum, University of California at Berkeley, California, U.S.A.; USNM for National Museum of Natural History, Washington, D.C., U.S.A.; and VAC, for Valeriu Albu Collection.

NEODACTRIA COCHISENSIS LANDRY & ALBU, SP. N. FIGS 1–9

Diagnosis. This species is similar in size and wing markings to smaller specimens of some of *N. luteolella* (Clemens) populations, but the forewing ground colour appears grey and the markings include a paler longitudinal streak in the cell and a darker diagonal dash from end of cell to apex. The male genitalia, especially the shape of the costal process of the valva, will readily separate this species from other members of the genus. In female genitalia, the unusually short anterior apophyses and the cylindrical shape of the corpus bursae are diagnostic.

Material examined: Holotype ♂ (Fig. 1): 1- 'Legit V[aleriu] M[agdalena] S[ebastian] A[lexander] Albu | Cochise Co./Arizona | Ramsey Canyon | 17 – 21 VII 2011' [printed black on white card stock]; 2- 'HOLOTYPE | *Neodactria* | *cochisensis* | Landry & Albu' [hand-written in black ink on red card stock]. Head and thorax partly rubbed, with appendages complete, not dissected, generally darker than paratypes. Deposited in the USNM. **Paratypes:** 3 ♂, 1 ♀; 2 ♂ (one with genitalia on slide BL 1780), with same data as holotype (VAC); 1 ♂ (genitalia slide BL 163), USA, AZ, Huachuca M[oun]t[ain]s, Ash C[an]y[o]n R[oa]d, 1550 m, 2.viii.1989, M[ercury]V[apour]L[ight] (B. & J.-F. Landry) (MHNG); 1 ♀ (genitalia slide BL 1369), Ariz., Cochise CO., Turkey Cr[eek]., 5600' [1707 m], Chiricahua Mtns., 1-2.viii.[19]86, b[lack] l[ight] trap (J. Brown) (UCB).

Description. MALE (n=4). (Fig. 1). Head greyish brown, with scales often tricolored, greyish brown in middle and paler at bases and tips, to whitish beige. Labial palpus slightly less than 4 times as long as widest diameter of compound eye; mottled greyish brown with tricolored scales as on head, often with uniformly white scales, especially ventrally. Maxillary palpus mottled greyish brown with tricolored scales as on head, white medially. Proboscis scales white to whitish grey. Antenna with scape and



Figures 2-7. Genitalia of *Neodactria cochisensis* sp. n. 2. Male genitalia, slide BL 1780, without phallus. 3. Phallus (enlarged compared to Fig. 2). 4. Male genitalia in lateral view, same preparation, prior to mounting. 5. Same, ventral view. 6. Same, apical view. 7. Female genitalia, slide BL1369, ventral view.

pedicel white and greyish brown; flagellum whitish grey. Thorax mottled greyish brown with tricolored scales as on head, with more uniformly paler whitish grey and lustrous scales on tegulae. Foreleg laterally dark greyish brown, darker at tip of coxa, beige on epiphysis, whitish grey at tips of tarsomeres; mostly white medially except for pale greyish brown tarsomeres III-V. Midleg as foreleg but laterally paler. Hindleg paler still, with tibia and first tarsomeres almost entirely snow white. Forewing length 6.5-7.0 mm (holotype: 7.0 mm); forewing colour mostly mottled greyish brown, with bi- or tricoloured scales as on head; median and subterminal lines warmer brown and most conspicuous between

M_1 and CuA_2 , paler, greyish brown with light yellowish scales between CuA_2 and inner margin; additional markings as a white streak in cell and until subterminal line, sometimes with darker scales especially distally, a dark brown dash from end of cell to apex, often interrupted after subterminal line, a yellowish-beige line along cubital stem and CuA_2 , often less conspicuous distally, light silver lustrous scales as small patches above yellowish line on distal half and along subterminal line posteriorly, and thin dark brown terminal line from apex to above tornus; fringe lustrous with basal row of shorter scales brown at apex, white with two pale greyish-white spots until M_3 , pale greyish-white until tornus,



Figures 8, 9. Photographs of collecting localities of *Neodactria cochisensis* sp. n. 8. Old Homestead and surrounding vegetation in Ramsey Canyon Preserve at 1676 m in elevation and at 1100 m southwest of type locality (Photo by Sebastian Albu). 9. Ash Canyon at paratype locality (Photo by J.-F. Landry).

with second row of longer scales pale greyish brown. Hindwing greyish brown; fringe with pale scales, with basal row of shorter scales pale greyish white, with second row of longer scales paler, dirty white. Abdomen dorsally dark greyish brown on first two segments, gradually paler to greyish beige around genitalia; ventrally paler, with white on basal segments. Tympanal organs (n=3): Similar to those of *Neodactria luteolella* (Clemens) (see Landry, 1995: p. 198 fig. 230) except for shorter tympanic drums, not reaching tympanic bridge.

Male genitalia (n=2). (Figs 2–6). Uncus about as long as tegumen, slightly down curved evenly, evenly thin in side view, only slightly compressed from base to apex in dorsal view, with moderate setation from after base to before apex, apically rounded. Gnathos poorly developed, with very thin arms directed anteroventrally, apparently not connected ventrally. Tegumen short and bulky, with lateral arms about as long as dorsal connection, ventral connection narrow, apparently complete. Valva with costal process a wide hook well separated from costa of cucullus, reaching about middle of valve, slightly bulging dorsally at about 3/4, apically narrowing and pointed, like a Great Northern Loon's (*Gavia immer*) head, projecting medioventrally, with moderate setation mainly along dorsal edge; cucullus constricting at right angle and then half right angle from ventral margin at 2/3, with costal margin nearly straight, slightly upturned in distal 1/3, with ventral margin broadly concave at 1/3, apically rounded, with abundant, short setation along ventral edge, medially and along costal margin less abundant but longer; sacculus forming more thickly sclerotized and apically rounded short ridge with moderate to short setation medially. Juxta associated with large membranous sac reaching inward beyond vinculum to distance equal to length of vinculum. Vinculum with arms of moderate width, only slightly longer ventrally, with medioventral margin straight, without saccus. Pseudosaccus low and short, reaching middle of vinculum's ventral length. Phallus straight, about 10% longer than valva, open dorsally on distal 1/3 and slightly directed downward, with scobination on ventral wall on distal third, apically with thin, pointed projection; vesica without cornutus.

FEMALE. (n=1). Forewing length: 8.5 mm; frenulum not visible (specimen not spread). Female genitalia (n=1) (Fig. 7): Papillae anales with setation of medium length and with scobination. Posterior apophyses about as long as papillae, straight, moderately narrow to very narrow distally, apically blunt. Anterior apophyses very short, subtriangular. Segment VIII very narrow dorsally, unsclerotized medially. Sterigma a pair of ventral rounded lobes of medium size reaching beyond posterior margin of segment, associated with short, membranous, rounded pouch. Ductus bursae short, about 1/3rd length of corpus bursae, of medium girth, doubling in size at midlength. Corpus bursae cylindrical and long, about 1/5th longer than segment VII, distally rounded, without signum.

Distribution. So far as known this species is only found in the Huachuca and Chiricahua Mountains, in Cochise County, Arizona.

Natural history. Unknown except that the moths are in flight between mid-July and early August and come to light. This species is probably univoltine given the strong seasonality of the rains in Arizona and also given that *N. caliginosellus* (Clemens, 1860), the only *Neodactria* species for which this information is known, is univoltine in Michigan and Virginia (Tashiro, 1970). The habitats in which this species has been collected are located between 1550 and 1707 m. The elevation at the type locality is about 1615 m and the vegetation is a blend of moisture loving sycamores (*Platanus* spp., *Platanaceae*), maples (*Acer* spp., *Sapindaceae*) and columbines (*Aquilegia* spp., *Ranunculaceae*) along the stream that flows through the canyon with extensive pine (*Pinus* spp., *Pinaceae*) and oak (*Quercus* spp., *Fagaceae*) forests (Fig. 8) on the dry slopes to desert grasslands at the bottom. Cacti (*Cactaceae*), yucca and agave (*Yucca* spp. and *Agave* spp., *Asparagaceae*) plants

abound at this elevation. Figure 9 shows some of the vegetation at the Ash Canyon locality when the paratype was collected, in 1989. Caterpillars should be looked for at the base of, including below ground, grasses or other plants in the Poaceae family as other species of *Neodactria* have mostly been reported to feed on grasses, sometimes to the point of becoming pests of lawns, and seedling corn, but also on narrow leaf plantain (*Plantago lanceolata* L., *Plantaginaceae*) (see references in Landry, 1995: 103).

REMARKS

The species' name is derived from the Arizona County where all known specimens were found. The county name in turn is derived from that of the legendary Chiricahua Apache war chief Cochise (ca. 1805–1874). The only female specimen available was not collected along any of the males nor in the same mountain range, but the markings are the same as in the available males, so much so that we are confident that the sexes of this species are not wrongly associated. The female genitalia of the unique female available were mounted on slide long before their description, which therefore is incomplete regarding some details. The type locality, in Ramsey Canyon, as well as Ash Canyon, are situated in the Huachuca Mountains, about 106 km west and south of Turkey Creek in the Chiricahua Mountains. The Ash Canyon specimen was collected on Noel McFarland's property, which was completely devastated in the fire that swept through the region in 2011. Only one other species of *Neodactria*, *N. luteolellus* (Clemens) is known from Arizona as far as we know. A series of 49 specimens collected in the White Mountains (some with additional locality data such as 'near McNary P[ost?]. O[ffice?]', 'near Rice, Elev[ation]. 7000 ft', 'Apache Ind[ian]. Res[ervation]. Elev. 7000 ft') in July and August 1925 by O. C. Poling (USNM) was examined and a pair was dissected to confirm the identification. This area is located about 240 km to the north of the Chiricahua Mountains locality, where a paratype of the new species was collected, and 294 km from its type locality. The higher elevation of this area (2133 m) and its more northern situation may mean that its habitats differ significantly from the habitats in which the new species was found. The forewing of these specimens is ochre brown at the base and usually darker brown on distal half, with the transverse bands sometimes indistinct. Their forewing length is between 10 and 12 mm. Their genitalia are as illustrated by Landry (1995: figs 283, 330), except for the less elongate and more angled tegumen in the male and the medially constricted corpus bursae in the female.

The discovery of *Neodactria cochisensis* sp. n. is not especially surprising as there are still too few collectors of smaller moths working in the vast species-rich parts of the south-western U.S.A. and taxonomists

interested in these moths are also very scarce. Since the works of Alexander B. Klots on North American Crambinae, between 1940 and 1970, when he described 25 species or subspecies, only two genera and eight species of Crambinae have been described from North America, all but one species by BL and co-authors, or as single author. However, a few undescribed species of Crambinae genera other than *Neodactria* are known from collection specimens by BL. The restricted distribution of this new species in only three localities of south-eastern Arizona is not entirely surprising either as the last three species described in *Neodactria* are also known from restricted ranges: *N. daemonis* Landry & Klots, 2005 from one locality in Arkansas and one in Missouri, *N. oktibbeha* Landry & Brown, 2005 from two localities in Mississippi, and *N. glenni* Landry & Klots, 2002 from prairie sites in upper central Illinois, central eastern Mississippi, and central and east-central Missouri. However, the known restricted range is again partly a reflection of the poor sampling of the south-western sky islands, especially in adjacent Mexico.

ACKNOWLEDGEMENTS

BL thanks Jean-François Landry and Yves Bousquet, both affiliated with the Canadian National Collection of Insects, Ottawa, for their company during the trip to Arizona that allowed him to collect a specimen of the new species described here. During this trip, the hospitality of Noel McFarland and his family was greatly appreciated. BL also thanks J.-F. Landry for the use of his photo, shown here as Fig. 9, and C. Ferris and J. A. Powell for answering specimen requests. VA thanks his sons Sebastian and Alexander Albu for their company in the field and for the habitat photos. And we both thank the reviewers and editor for their gracious and useful comments on the manuscript, and for their alacrity.

EDITOR'S NOTE

The electronic edition of this article has been registered in ZooBank to comply with the requirements of the amended International Code of Zoological Nomenclature (ICZN). This registration makes this work available from its electronic edition. ZooBank is the official registry of Zoological Nomenclature according to the ICZN and works with Life Science Identifiers (LSIDs). The LSID for this article is: urn:lsid:zoobank.org:pub:E0DD0AF4-9FC5-4F4A-8591-9913A4C25852. Registration date: December 28th, 2012. This record can be viewed using any standard web browser by clicking on the LSID above.

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The bionomics of *Spindasis greeni* Heron, 1896 and a review of the early stages of the genus *Spindasis* in Sri Lanka (Lepidoptera: Lycaenidae)

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Abstract. The occurrence of *Spindasis greeni* in Sri Lanka and its status as a species is confirmed more than 100 years after the original description. Additional descriptive notes of the male based on fresh material are presented. The female, the immature stages and behavior are documented for the first time and its distribution mapped. For comparison, the immature stages of *S. vulcanus fusca*, *S. ictis ceylonica* and *S. elima fairliei* in Sri Lanka are described for the first time and their larval food plants and ant associations identified.

Keywords: *Spindasis*, immature stages, Sri Lanka, Lycaenidae, ant associations, *Crematogaster*, *Tapinoma*.

INTRODUCTION

Heron (1896) described *Spindasis greeni* (subfamily Theclinae, tribe Aphnaeini) from a single, worn specimen of a male obtained by E. E. Green in Sri Lanka. It was accepted as a valid species by some authors but not by others. de Nicéville & Manders (1899) listed it as *Aphnaeus greeni* but noted that de Nicéville had examined the specimen and preferred to express no opinion regarding its validity as a distinct species. Swinhoe (1911–1912) listed it as *A. greeni* and re-described it from the holotype. Ormiston (1918) listed it as *A. greeni* and stated that Evans thought it was an aberration of *A. [vulcanus] fusca*. Ormiston (1924) listed it as *S. greeni* (along with the synonym *S. lunulifera* ab. *greeni* Riley) and stated that Riley would not create a new species in this genus from a single specimen because aberrations are so common in the genus. He further stated that the genitalia prove its connection with *S. lunulifera* but did not provide any description or illustration to support this statement. Evans (1927, 1932) and Woodhouse (1949) thought it

was *S. ictis ceylanica* [sic]. d'Abrera (1986) wrote “I am not certain that this is a ‘good’ species. The unique holotype (male) is probably an aberration of another local species – precisely which species is hard to tell because both surfaces are so weakly marked.” d'Abrera (1998) did not list it. H. Gaonkar (pers. comm.) listed it as an endemic species for Sri Lanka despite some reservations since he also noted “The type - (male) HOLOTYPE - of this species is unique. I am uncertain whether the specimen actually belongs to a distinct species described as *greeni* by Heron or an aberration of another species. New materials will be needed.”

Seven species of *Spindasis* are reported from Sri Lanka: *S. vulcanus fusca* (Moore, 1881), *S. schistacea* (Moore, 1881), *S. nubilus* (Moore, [1887]), *S. ictis ceylonica* Felder, 1868, *S. elima fairliei* Ormiston, 1924, *S. lohita lazularia* (Moore, 1881), and *S. greeni*. All are endemic at least at the subspecies level except for *S. schistacea* and *S. lohita lazularia*. Fourteen species of *Spindasis* and two related *Apharitis* species are found in India, to which Sri Lanka is zoogeographically related: (1) *Apharitis acamas hypargyros* (Butler, 1886), (2) *A. l. lilacinus* (Moore, 1884) (3) *Spindasis abnormis* (Moore, 1884), (4) *S. e. elima* (Moore, 1877), *S. e. uniformis* (Moore, 1882), (5) *S. elwesi* Evans, 1925, (6) *S. e. evansii* (Tytler, 1915), (7) *S. gabriel* (Swinhoe, 1912), (8) *S. i. ictis* (Hewitson, 1865), (9) *S. maximus* (Elwes, 1893), (10) *S. nipalicus* (Moore, 1884), (11) *S. lohita lazularia* (Moore, 1881), *S. l. himalayanus* (Moore, 1884), *S. l. zoilus* (Moore, 1877), (12) *S. r. rukma* (de Nicéville, 1888), (13) *S. rukmini* (de Nicéville, 1888), (14) *S. schistacea* (Moore, 1881), (15) *S. syama peguanus* (Moore, 1884) and (16) *S. v. vulcanus* (Fabricius, 1775) (provisional list supplied by K. Kunte, pers. comm.).

Received: 9 May 2012

Accepted: 9 December 2012

Many Lycaenidae are known to exhibit larval-ant associations (Fiedler, 2006; Pierce *et al.*, 2002). In India and Sri Lanka, the members of the genus *Spindasis* are also considered to have similar associations (Woodhouse, 1949). However, of the 16 species of *Spindasis* recorded from these two countries, the early stages of only 3 species have been recorded: *S. lohita* (Marshall & de Nicéville, 1890; Green, 1902; Kershaw, 1907; Bell, 1919; Igarashi & Fukuda, 2000), *S. vulcanus* (Bell, 1919; Sidhu, 2010) and *S. abnormis* (Bean, 1968). The larva of *S. lohita* is reported to often build shelters (Kershaw, 1907; Bell 1919) and to often feed at night (Kershaw, 1907; Green, 1902; Igarashi & Fukuda, 2000). In the larval and pupal stages, all three species are associated with *Crematogaster* ants and additionally, with a species of *Pheidole* for *S. vulcanus* (Marshall & de Nicéville, 1890). The nature of the association, whether obligate or facultative, has not been reported in sufficient detail. As described in the current paper, the larvae of some species can reach the adult stage successfully in the lab without attendant ants. We speculate though that the presence of ants is necessary in the field to elicit oviposition by the female as has been shown for some other species (Pierce *et al.*, 2002). Each of these three species was recorded feeding on the leaves of more than one species of plant, sometimes from widely different families. This dependence on ants has evolved in at least one species to aphytophagy: *S. takanonis*, which is found in Japan, is completely aphytophagous, depending on its attendant *Crematogaster* ants to feed it mouth to mouth (Igarashi & Fukuda, 2000). Overall, the tribe Aphnaeini is known as a clade whose component species have particularly tight relationships with ants (Heath, 1997).

In April 2008, Nadeera Weerasinghe took several photographs of a butterfly ovipositing on a dead tree at the top of World's End in Horton Plains (Fig. 1a). Some photographs were forwarded to the authors who immediately recognized that it was an image of *S. greeni*. This was only the second sighting ever of *S. greeni*. A first attempt to locate the butterfly immediately after this did not succeed, but in March 2012 we were able to return to the location and located several males, females, eggs, pupae and a single larva.

MORPHOLOGICAL EVIDENCE FOR SPECIFIC DISTINCTNESS

Specimens examined

Male holotype in NHM (London): [Label: Type/ Pundalaya [Feb.] G.E. Green 91-150./30/Pdo. 2/ [blank blue label] (Figs. 1b, c). Additional new

specimens: 13 iii 2008 – 1♀, World's End (6.78092N, 80.79423E, 2100 m asl), Horton Plains; 24 iii 2012 – 1♂, 1♀ mating pair, Road B508, km 28 (6.79769N, 80.83039E, 2100 m asl), Horton Plains; 24 iii 2012 – 1♂, mini-World's End (6.78859N, 80.80174E, 2090 m asl), Horton Plains; 25 iii 2012 – 1♂, mini-World's End, 1♂, 1♀ mating pair, between mini-World's End and World's End (6.7288N, 80.79659E), Horton Plains. 1♂ and 1♀ to be deposited in the Sri Lanka National Museum.

Where information on the duration of developmental stages is given, these data were obtained in rearings at ambient temperatures (25–31°C) at Bandarakoswatte (07.37.01N, 80.10.57E), 70 m asl, North Western Province, Sri Lanka. Conventions used (applied to both the larva and the pupa): Segments are numbered S1 to S14 (S1—the head; S2 to S4—the 3 segments of the thorax; S5 to S14—the 10 segments of the abdomen). In the photographs, the head is on the left.

Comparison of newly collected males to the holotype

The somewhat worn holotype male was described by Heron (1896) and re-described by Swinhoe (1911–1912), and though both authors described the abdomen, it is now missing from the specimen. The newly acquired fresh specimens agree with these descriptions except for the following points: a) Forewing upperside: band along termen narrow at the tornus and wider at the apex, and continues more narrowly along the costa; band black or violet-brown depending on angle of view; color of remainder of wing depends on the angle of view—ranging from pale grayish blue to bright iridescent blue (Fig. 1d) to purplish blue (Fig. 1e); in all views, there are sparsely scattered pale grayish-blue scales from the base of the wing to the median line (Heron described them as “pale lilacine blue scales”); discocellulars and the radius clothed with black scales; dorsum lined with much longer pale brown hairs than the termen where the cilia are more densely packed. b) Hindwing upperside: color of iridescence between veins Rs and CuA₂ depends on the angle of view and varies from purplish-blue to bluish-gray (Fig. 1e); brownish-gray above vein Rs and below CuA₂; clothed at base and discal area with long pale brown hairs similar to those on the dorsum of forewing; anal fold densely packed with similar hairs. c) Forewing underside: ground color pale brownish-pink [not ochreous brown] with a pearly sheen; markings reduced but all are speckled with silver (Heron reported few); submarginal spots clearly visible (not traces) but diffuse. d) Hindwing

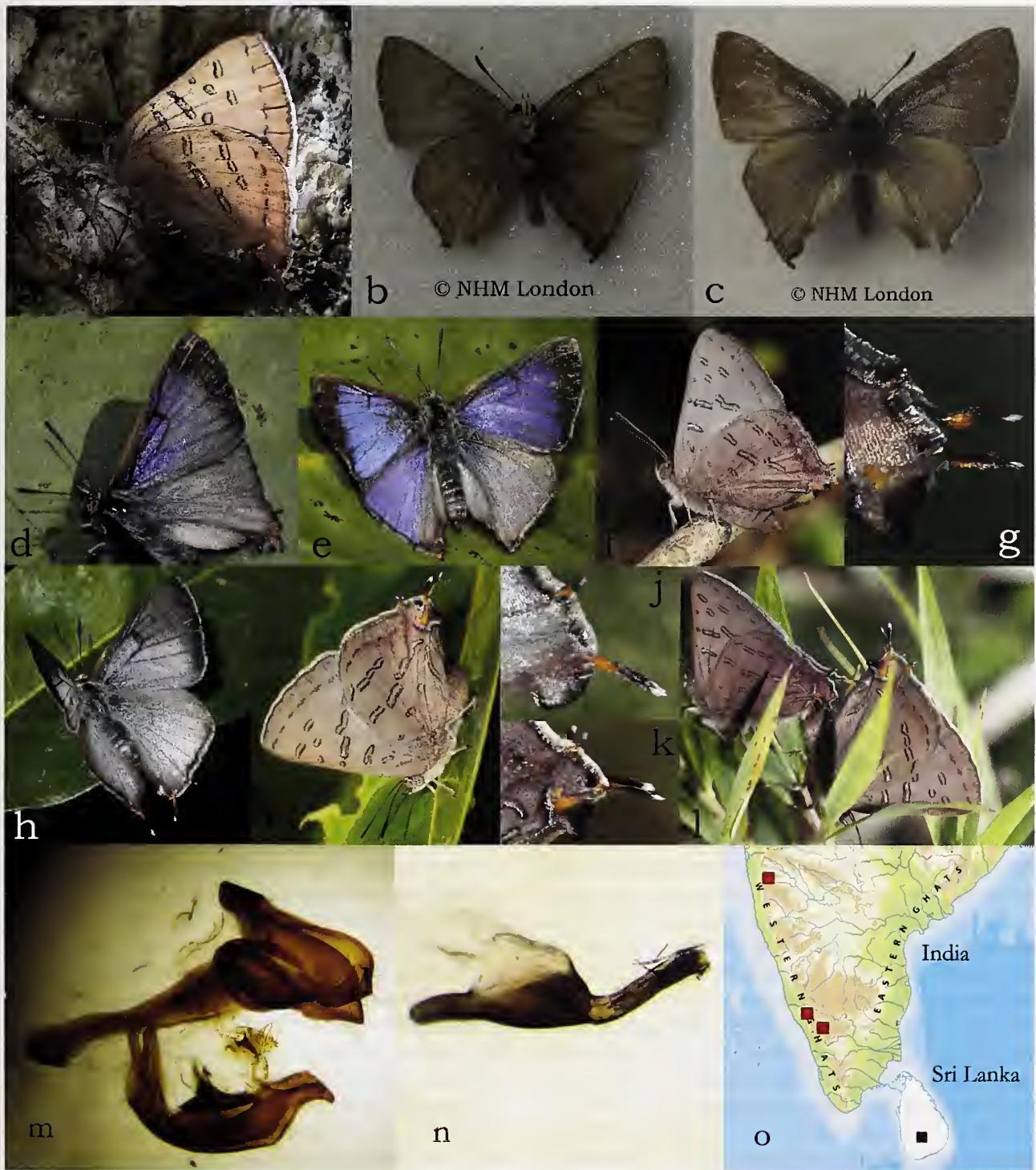


Figure 1. Adult *S. greeni* butterflies from Sri Lanka. **a)** Female, ovipositing on dead tree. **b)** Male, holotype, underside. **c)** Male, holotype, upperside. **d)** Male, upperside. **e)** Male, upperside. **f)** Male, underside, tails missing. **g)** Male, close up of tails, underside. **h)** Female, upperside. **i)** Female, underside, typical sitting posture. **j)** Female, close-up of tails, upperside. **k)** Female, close-up of tails, underside. **l)** Mating pair, male on the left. **m)** Male genitalia with aedeagus removed, lateral view. **n)** Aedeagus, lateral view. **o)** Distribution map of *S. abnormis* in India (red squares) and of *S. greeni* in Sri Lanka (black square).

underside: band between discal and submarginal bands clearly marked, not obscure; ground color darker shade of the forewing underside (brownish-pink); all markings with silver; small orange patch at base of each tail, often obscure or missing on the shorter tail. In both forewing and hindwing, ground color somewhat darker distally and the silver markings within the bands variable in intensity. Thorax and abdomen: black dorsally with salmon-colored scales laterally and ventrally that match the ground color of the underside of the wings. Antennae: outside edge black with white spots and bright orange at the tip; inner edge same color as underside of hindwing (Fig. 1f). Tails: Heron did not describe any tails though Swinhoe noted that the tails were broken. There are two pairs of tails: 1) 1 pair at CuA_2 , 0.5 mm long, tip white, black in the middle, orange at base; 2) 1 pair at $1A+2A$, 2 mm long, similarly colored (Fig. 1g) (2 specimens measured, both same length).

Wingspan 24 mm; forewing length along the costa 14 mm (2 specimens measured, both the same). Heron recorded the wingspan of the holotype specimen as 35 mm and Swinhoe as 1-3/10 inch [33 mm]. Since the holotype male was recorded as being much larger than the specimens we obtained, we re-measured the holotype male and found it to be 28 mm apex to apex, and 15 mm right forewing length.

First description of the female

Termen of forewing slightly more concave than that of the male. Forewing upperside: Ground color light bluish-gray with a silvery iridescence, gradually grading into grayish-black at the apex and along the termen towards the tornus; discal cell and cells below M_3 densely covered with scales, less so above M_3 . Discocellulars and anterior portion of cubitus clothed with black scales which continue partway up veins M_1 , M_2 , M_3 and R_5 ; dorsum with fine long grayish-blue hairs decreasing in length towards tornus; cilia white with base gray; ground color changes depending on angle of light from bluish to grayish.

Hindwing upperside: entire wing iridescent light bluish-gray; area above vein Rs lightly dusted with bluish-gray scales; long light bluish-gray scales along the base of the wing, the discal cell and along the anal fold; the hairs in the anal fold near the tornus reddish-purple; diffuse marginal black line from veins Rs to CuA_2 ; two pairs of tails—1) 1 pair at CuA_2 , 1 mm long, 2) 1 pair at $1A+2A$, 3 mm long (2 specimens measured, both the same). Both pairs of tails tricolored as per the male; both pairs of tails longer than those of male (Figs. 1h, j). Forewing and hindwing underside: similar to the male though

markings appear to be less pronounced (Figs. 1i, k). Wingspan: 26 mm; forewing length 16 mm (2 specimens measured, both the same).

Differential diagnosis

Heron (1896) compared the male of *S. greeni* to the male of *S. abnormis* from the Western Ghats in India (Moore, 1883; Bean, 1968; Lovalekar *et al.*, 2011), the species to which he thought it is most similar in appearance. However, since he had only a single worn specimen, he was unsure of some of the morphological features. Our findings confirm the similarities (both taken at higher altitudes and with reduced markings) and differences (shape and color of wings, greatly reduced and interrupted markings on the underside in *S. greeni*) that Heron pointed out and also confirm that the male *S. greeni* does not have the large ochreous-brown anal lobe on the upperside of the hindwing that is so prominent in male *S. abnormis*.

Comparing specimens in the NHM London and the description in Swinhoe (1911–1912), we observed the following differences in the females of the two species: the female of *S. greeni* lacks the dull red anal lobe and the discal cell-end bar on the upperside of the hindwing which are present in female *S. abnormis*. As in the male, the markings on the underside are interrupted and significantly reduced in *S. greeni*. *S. abnormis* is considerably larger as reported by Moore (1883): ♂ 1.5 inches (38 mm) and by Swinhoe (1911–1912): ♂ 1.6 inches (40.6 mm) and ♀ 1.7 inches (43 mm).

The 5 males and 4 females of *S. greeni* observed in the field exhibited minimal variation. *S. greeni* is morphologically distinct from all other Sri Lankan *Spindasis*.

The genitalia of two males were examined and compared to those of *S. abnormis* described and illustrated by Bean (1968) and to Sri Lankan species of *Spindasis* illustrated by Woodhouse (1949). The genitalia of *S. greeni* (Figs. 1m, n) are distinct from *S. abnormis* in the following respects (in lateral view): 1) each lobe of the uncus is broader and more obtuse at the apex; 2) the costa of the valve is deeply curved (in *S. abnormis*, it is only slightly curved); 3) the apex of the valve is beak-like with 3 distinct protuberances along the dorsal edge (in *S. abnormis*, the valve is deeply indented on the dorsal edge to form a cleft posterior to the apex); 4) the supravzone of the aedeagus is distinctly bent upwards and slightly curved at the tip (the supravzone of *S. abnormis* is straight); 5) the subzone of the aedeagus is convex on the ventral margin (that of *S. abnormis* is straight); and 6) the vinculum is much narrower. The genitalia of *S. greeni*

are also distinct from all other Sri Lankan *Spindasis*, but are most similar to those of *S. elima fairliei* (which Ormiston referred to as *S. lunulifera*).

These observations show that *S. greeni* is not a subspecies of *S. abnormis* or a different phenotype of any other species of *Spindasis* as suggested by some authors. Given the morphological similarities between *S. greeni* and *S. abnormis* and the close zoogeographic relationship of the southwestern sector of Sri Lanka with the Western Ghats in India (Myers, 1988; Myers *et al.*, 2000), it is possible that they are closely related phylogenetically. Molecular studies are needed to test this idea.

Distribution and habitat

Horton Plains is located in the south-western sector of the island and though it has some affinity with the Western Ghats, it is also unique. The distribution of species pairs such as *S. abnormis* in the Western Ghats and *S. greeni* in Horton Plains is not unusual (Fig. 1o) since there are similar instances for other taxa. For example, in an analysis of the vascular plant flora, Gunatilleke & Pethiyagoda (2012) found that 9% of Sri Lanka's plant species and 12% of its endemics occur in Horton Plains and that 28% of the non-endemic species of Horton Plains are found only in Sri Lanka and the southern mountain ranges of the Western Ghats. Mani (1974) reported that the Turkmenian (Palaearctic) elements of the Indian flora and fauna were confined to the higher Himalayas and occur as isolates on the Eastern and Western Ghats and "even in the hills of Ceylon [Sri Lanka]." Among birds, there are several "species pairs": for example, *Saxicola caprata* is a breeding resident in Horton Plains, and is also found, along with several other species of *Saxicola*, in the Western Ghats. In butterflies, *Udara singalensis* and *U. lanka* are endemics that are found in Horton Plains, while the related *U. akasa* is found in Horton Plains as well as the Western Ghats.

Heron (1896) reported that the holotype male had been taken "near Pundaloya, on the summit of the great western range of hills in Ceylon, at this point attaining a height of about 6000 feet [1828 m]." He also listed "Hab." as "Pundaloya" which was repeated by subsequent authors. However, it is unlikely that Pundaloya (which is where Green was stationed) is the true location of the original specimen as it is located on a plateau at a much lower elevation (1050 m asl). However, near Pundaloya there is a mountain called the Great Western Mountain (6.96451N, 80.69321E) which reaches 2200 m asl at its peak. It is very likely that this is the location where Green caught the original specimen.



Figure 2. Habitat of *S. greeni* from Sri Lanka. **a)** First author at World's End, escarpment visible at left. **b)** Typical habitat with stunted vegetation; dead tree on which the larva and pupae were found to the right.

The sighting in 2008 was at World's End in Horton Plains. The sightings in 2012 were at World's End, mini-World's End and on the Ohiya Road (B508 at km 28) in Horton Plains (all at approximately 2100 m asl). The locations in Horton Plains are about 20 km crow's flight distance from the Great Western Mountain, and though they are part of the same mountain complex, they are not contiguous and are separated by a plateau with an elevation of about 1600 m asl.

Horton Plains is a unique habitat in Sri Lanka—it is a highland plateau at the southern edge of the central mountain massif, 31 km² in extent. On the south-eastern side, it is bounded by the World's End escarpment which is about 800 m high (Fig. 2a). Road B508 also runs along the edge of the escarpment. Rainfall is moderate (around 2000 mm per year); mean annual temperature is 15°C (range: 0 to 28°C). Cloudy, misty days prevail for most of the year and there are strong winds during the south-west monsoon (May to July). The vegetation comprises Tropical Montane Cloud Forest (80%) and Wet Patana Grasslands (20%), and the trees are generally stunted (about 12 m tall). *Callophylum walkeri*, *Syzygium rotundifolium*, *Symplocos elegans*, *Cinnamomum ovalifolium*, *Glochidion pycnocarpum* and *Neolitsea fuscata* are major tree components of the forest (Gunatilleke & Pethiyagoda, 2012; Gunatilleke *et al.*, 2008). Canopy die-back is an extensive problem in the park. Though first reported in 1978, the reason for the die-back is still unknown though several hypotheses have been advanced (Werner, 1988; Adikaram *et al.*, 2006). Fifty-five species of butterflies have been recorded from the park though only 23 species are considered to be resident (van der Poorten, 2012). The others simply fly through the plains during their migrations. *S. greeni* was found on the edge of the escarpment in areas with stunted vegetation and dead trees (Fig. 2b).

Though *S. greeni* has been reported from only Horton Plains and the Great Western Mountain, there are other locations in Sri Lanka that are also covered by Tropical Montane Cloud Forests (TMCF) (Gunatilleke & Pethiyagoda, 2012) where *S. greeni* might be found. Hakgala Strict Natural Reserve and Pedro Forest Reserve have a similar climate and vegetation to Horton Plains though there is no information as to whether or not the attending ant, *C. rothneyi* or another species of *Crematogaster* is found there. The Peak Wilderness Sanctuary above 1500 m asl, the Knuckles Conservation Area and the peak of Namunukula are also TMCFs (Gunatilleke & Pethiyagoda, 2012) but have slightly different climatic conditions and vegetation; again, there is no information about the ant species found there.

Faunistic data (sightings by the authors unless otherwise indicated): March 13, 2008 – 1 ♀ observed and photographed ovipositing on a dead tree at World's End in Horton Plains (N. Weerasinghe, pers. comm.); March 24, 2012 – 1 ♂ and two eggs at Mini-World's End (H. D. Jayasinghe & C. de Alwis, pers. comm.) and one mating pair on Ohiya Road (B508) at Km 28; March 25, 2012 (with H. D. Jayasinghe & C. de Alwis) – one mating pair, one larva and four pupae between Mini-Worlds' End and World's End; 1 ♂ at Mini-Worlds' End.

Adult behavior and associated ants

Like others in the genus, both sexes fly very rapidly and are difficult to follow once airborne. However, they frequently settle on the top of the stunted trees that are common in Horton Plains but seldom descend to the ground. They are best observed through binoculars. Once settled (often with the head pointing down), they frequently spread their wings open halfway and slant their bodies to bask in the sun to warm up. Frequent warming up is essential at these altitudes to keep body temperature optimal for flight—the ambient temperatures here are often well below 10°C in the mornings and seldom exceed 20°C.

Though no *S. greeni* butterflies were observed feeding on floral nectar, several plants such as *Ageratina riparia*, *Hedyotis lessertiana*, *Vernonia wightiana* and several species of *Knoxia* were in bloom at that time. In India, Bean (1968) recorded *S. abnormis* feeding on many different flowers.

Courtship was not observed but two mating pairs were seen (Fig. 11). In both cases, both individuals were in pristine condition and had presumably just emerged. When the pair in copula was disturbed, they flew together to a point just a few meters away. They repeated this process of flying and settling a few more times.



Figure 3. *Crematogaster rothneyi*: ants associated with *S. greeni* in Sri Lanka. a) Worker ant. b) Ants with their brood.

A female was observed ovipositing on the bark of a dead tree in March 2008. On March 25, 2012, eggs were also found on the bark of a dead tree, in a small crevice but still visible from the outside. Eggs were laid singly or in batches of 2–3 and were found only on trees that harbored the ant *Crematogaster rothneyi* Mayr, 1878 (Formicidae: Myrmicinae) (Figs. 3a, b). It is not known how the female is able to determine the presence of ants because not all dead trees harbor a colony and ants were not visible on the surface. No ants were observed attending the eggs.

There are 13 species of *Crematogaster* recorded from Sri Lanka (Dias, 2002). *Crematogaster rothneyi* is widespread in southern Asia and has been reported from all over India (Bingham, 1903) (*C. r. rothneyi*, *C. r. cava* Forel, 1902), from Haputale in Sri Lanka (*C. rothneyi haputaleensis* Forel, 1913) (Forel, 1913) and from Kampong Chnang in central Cambodia (antweb.org, 2012; subspecies not identified), from Rangoon, Burma (Wheeler 1927; subspecies not identified) and from the Punjab province in Pakistan (Umair *et al.*, 2012). The majority of *Crematogaster* species in India build brown papery nests made of vegetable fiber on a large tree but some species build nests in hollows of trees, in the ground or under stones. The same species may build a different kind of nest in different parts of the country (Bingham, 1903). In India, *C. rothneyi* has been reported as nesting in soil, in crevices in the walls of buildings (Ayyar, 1937) or as nesting in a “carton” nest (presumably a brown papery nest) on the trunk of a large tree in Tamil Nadu (Tiwari, 1999). In Sri Lanka, the distribution of *C. rothneyi* is unknown though the type of *C. rothneyi haputaleensis* was recorded from Haputale at 1500 m asl. There are no reports of its nesting habits.

In this study, the ants were found in a large colony, inhabiting dead trees where they used the galleries already established by other insects, possibly coleopterans. No ants were visible on the surface of the tree but when the tree was pounded upon quite

vigorously many ants moved out to the surface of the bark. Underneath the bark, a colony was visible with a large number of ant eggs, larvae, pupae and workers in the crevices. Of the 8 stumps examined, a large colony of *C. rothneyi* was found in 5 of them and no other ants were observed. There is no information on the ant fauna of Horton Plains and the ecology of *C. rothneyi* is not known. Umair *et al.* (2012) reported that it was found in Pakistan in grasslands, fields of maize and wheat, apple orchards and pine trees. It appears to be an ecologically dominant species where it occurs.

Immature stages of *S. greeni*

Egg: pale green when first laid, turning beige within one day and darker brown just before hatching, dome-shaped and finely sculptured with hexagonal and pentagonal depressions, somewhat reminiscent of the surface of a golf ball, similar to eggs of other species of *Spindasis* (Fig. 4a). 1st instar: ate only top portion of eggshell; larva very active; length 3 mm; head black, not retracted; body ground color light purplish-pink, an indistinct light bluish-gray band dorsally fading towards the posterior end and absent beyond S10, S2–S3 dark purple dorsally and lighter colored laterally, S4–S7 with more or less oval spots on either side of the dorsal line embedded within the dorsal band, S2–S14 with long silvery-pink hairs laterally, 2 per segment from S4–S12, more numerous on anterior segments and anal plate. Dorsal nectar organ (DNO) on S11 appears as a darker reddish slit, S12 with a pair of rudimentary tentacle organs (TO), anal plate grayish-pink. Mouthparts normal (Fig. 4b). 2nd–4th instars not seen.

Final instar, prepupation: typical *Spindasis* shape; length 16 mm; head black, not retracted; body pale yellowish-brown, dorsal line dark reddish-brown, an obscure more or less discontinuous reddish-brown subdorsal line, lateral line of similar color but broader and more distinct, dorso-ventral flange pink, tufts of setae at the center of each segment along the flange, DNO on S11, a pair of TOs on S12 with white rod-like eversible tentacles, opening of the tentacles lined with black hairs, dark brown patch on dorsum of anal plate surrounded by a ring of setae, spiracles obscure and small, yellowish-white, anterior edge of S2 with a series of fine long hairs, dorsum of S2 with a rhomboidal depression that is darker than the surroundings (Figs. 4c–e). The larva just described was found underneath the bark of a dead tree with ants attending the whole body, indicating that the larva likely has pore cupola organs (Fig. 4f).

Pupa: typical shape of *Spindasis*, length 12–13.5 mm, width 3–5 mm; smooth, head pale cream-

colored, prothorax reddish-brown with two dark rings (appearing like eyes) on the dorsum, mesothorax dark brown dorsally fading to a lighter shade subdorsally and laterally, metathorax lighter reddish-brown, wing buds and abdomen light brownish-yellow but darker on the dorsum, spiracles small, slit-like and brownish-red, S8–S11 with obscure dark circular areas surrounding the spiracles (Figs. 4g, h). Some pupae lighter colored (Figs. 4i, j). Attached by cremaster only, no girdle; weakly attached to the substrate. In the field, pupae were found on the ground under fallen debris and just underneath the bark in close proximity to an exit hole with the head pointing down. Ants also attended the pupa that was underneath the bark (Fig. 4k).

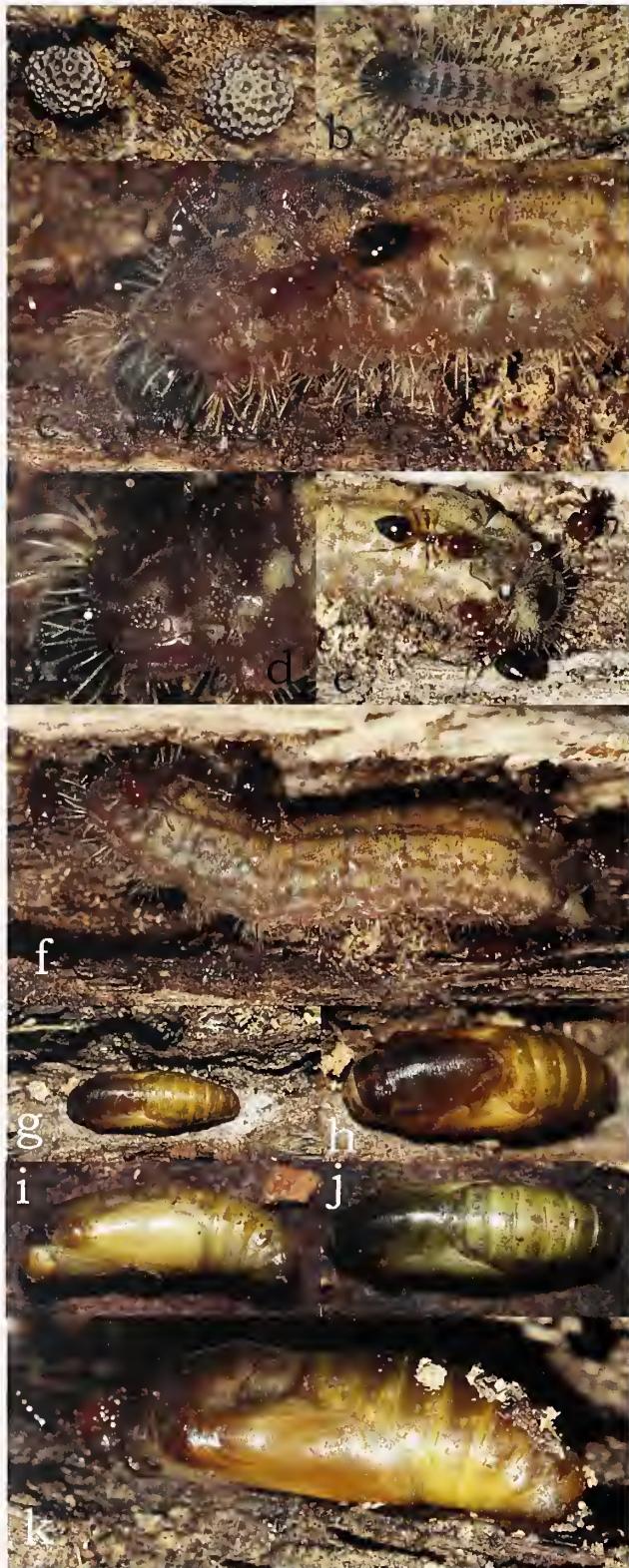
Duration of immature stages: egg more than 7 d; pupa more than 7 d.

Some thoughts about larval life-history of *S. greeni*

Though we have not been able to identify the exact association that *S. greeni* has with ants and have not been able to document the immature stages completely, we present the following information and speculations.

Several eggs and a single final instar larva were found in the field on dead trees within 1–2 m from the ground (heights above this were not examined); these dead trees all had colonies of *C. rothneyi* and moist decaying tissue within. In one of the trees examined, a larva was found just underneath the bark. After a few photographs of it were taken *in situ*, the larva moved and dropped into the dense undergrowth and could not be recovered for further study. No eggs or larvae were found on dead tree trunks that had dry, hard wood inside despite the presence of ants. During oviposition, the ants were not observed on the outside of the bark of the dead trees and we have not been able to determine how the adult female locates the presence of ants. The most likely mechanism is that the female (as has been shown in other obligate myrmecophiles) is able to recognize some of the many pheromones used by their host ants, and then finds trees with ant colonies (K. Fiedler, pers. comm.).

In the lab, we opened up a piece of dead wood to expose the ants and ant galleries and placed newly hatched larvae on the wood. The larvae moved very quickly from the surface, away from the bright light, into an ant gallery which was 2–3 mm wide. While moving, the larvae had no reaction to the ants present nor the ants to the larvae; both parties ignored each other. It was not possible to retrieve the larvae or make any records of them once they entered the dead wood, and careful inspection of the wood after 4 and 10 days did not reveal any larvae. We presume that they had died.



In the field, we observed ants attending the final instar larva and pupa, just underneath the bark within the galleries, but the exact nature of the relationship is not clear. The larva was attended by 5–10 ants which were spread over the entire body, but often concentrated around the DNO and the junction of S2–S3 from which the ants seemed to derive some nutriment. None of the ants were moving about excitedly as in the case of *S. vulcanus fusca* (see below) or as with *S. abnormis* (Bean, 1968) but were decidedly subdued. When the ants were near the DNO, the larva frequently everted the 2 TOs but no response from the ants was observed (though in other *Spindasis* such as *S. vulcanus fusca* this often excites the ants). The tree on which the larva was found had large (10–20 mm diameter) interconnected passageways that were of sufficient diameter for the movement of the larger, later instar larvae of *S. greeni* to forage and rest. However, the ant passages, which were connected to these larger passages, were smaller and would not have allowed a full grown larva to enter or exit. This shows that a later instar larva would not have access to the ant brood on which it may possibly feed. It is possible that the larva feeds on the ant brood in the early stages when it can move in and out of the brood chambers easily. However, in the lab, the first instar larvae paid no attention to the ant brood that was offered to them when they first emerged.

Another possibility is that the ants feed the larva mouth to mouth through trophallaxis, as has been reported for *S. takanonis* in Japan (Igarashi & Fukuda, 2000) or that the larva of *S. greeni* feeds on vegetation outside the dead tree. Other aphytophagous lycaenids have also been recorded as feeding on honeydew (Cottrell, 1984) or hemipterans. Further investigation is required to elucidate the feeding behavior of the larva of *S. greeni* and its association with the ants.

Review of the immature stages of species in the genus *Spindasis* in Sri Lanka

In addition to *S. greeni*, there are 6 other species of *Spindasis* in Sri Lanka. All members of the

Figure 4. Early stages of *Spindasis greeni* from Sri Lanka. **a)** Eggs. **b)** Larva, first instar. **c)** Larva, final instar, prepupation, anterior end, attending ants visible. **d)** Larva, as in (c), close up of S2–S3. **e)** Larva, as in (c), posterior end, attending ants visible. **f)** Larva, as in (c), attended by ants; photo rotated 90° counterclockwise from its natural position. **g)** Pupa, dorsal view. **h)** Pupa, same as (g), close-up. **i)** Pupa, lighter colored, dorsal view. **j)** Pupa, same as (i), lateral view. **k)** Pupa, *in situ* underneath bark, attended by ants; photo rotated 90° clockwise from its natural position.

tribe Aphnaeini whose life-histories are known are myrmecophiles and almost all obligately so (K. Fiedler, pers. comm.). Heath (1997) reported over 60 species of *Chrysoritis* from Africa with such an association. In Asia, only 3 species of *Spindasis* have been reasonably well-studied (*S. lohita*, *S. syama* and *S. takanonis*) and they also appear to be obligate myrmecophiles. However, the ant association has not always been carefully studied. For example, Bell (1919) wrote of *S. vulcanus* in India, "The eggs are laid anywhere...on practically any plant where there are ants of the genus *Crematogaster*—a particular species probably. The ants look after the little larvae from the first and these do not get on well without them." However, the results of our studies on Sri Lankan populations detailed below differ from those presented by Bell.

Spindasis vulcanus fusca (Moore, 1881). Common Silverline.

The immature stages of *S. vulcanus fusca* in Sri Lanka have not been described. In India, the final instar larva and pupa of *S. vulcanus* were described by de Nicéville (1890) and by Bell (1919), later quoted by Woodhouse (1949) and by Sidhu (2010). The results of our observations agree with these descriptions except for the following points: a) the larva is widest at S6–S7 (not S4), the constrictions between the segments are quite visible and the dorsal depressions are found only on S5–S8 (not S2–S11) (Fig. 5a); b) pupa (11 mm long, 4 mm wide) light green or black (Figs. 5b, c).

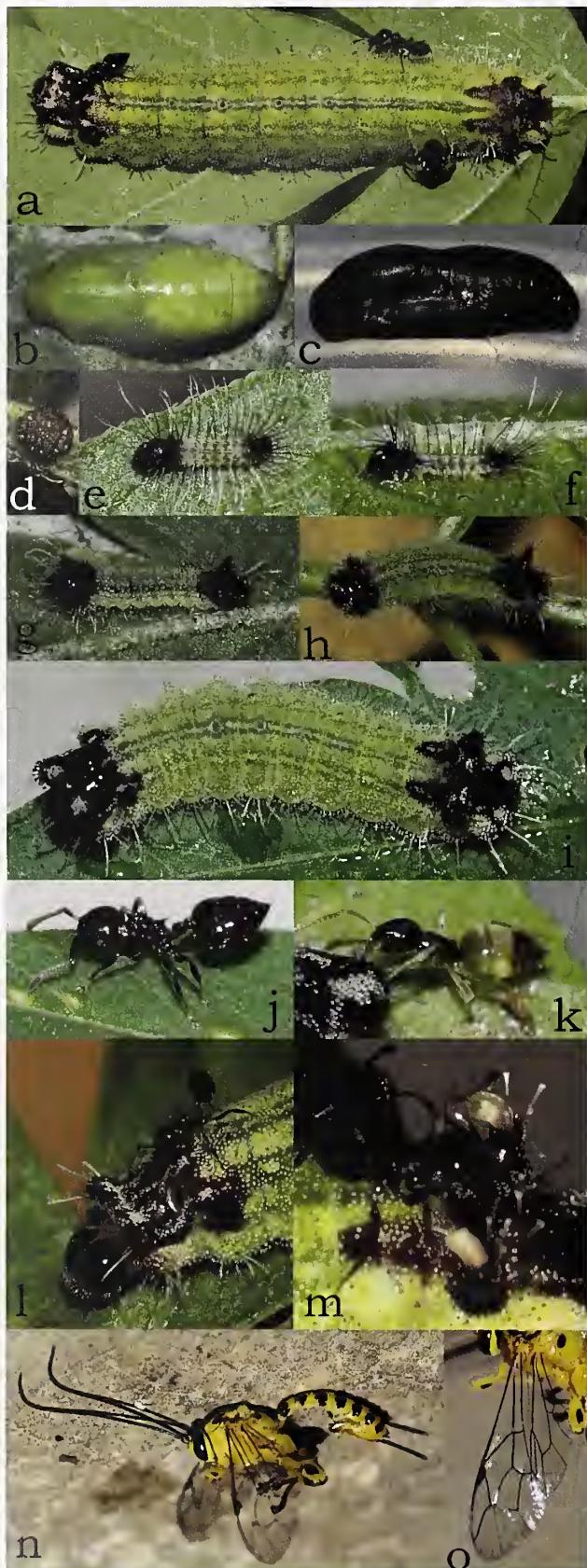
Egg laid singly on twigs, leaves and buds of the larval food plant, turns purplish-brown after a few days (Fig. 5d). 1st instar: newly emerged larva—head black and rather large, body light gray to pale yellow with two discontinuous dorsal grayish-brown lines on S7–S9 and darker colored patches of similar color subdorsally, S2–S4 and S10–S14 dark purple with long setae, in the remaining segments setae confined to the lateral margin (Figs. 5e, f). 2nd: almost identical to 1st except that body is light green and the subdorsal band is diffuse and a darker green, 3–4 small black subdorsal spots on each segment, S2–S4 and S10–S14 darker purple (Fig. 5g). 3rd: similar to 2nd but subdorsal band now with a whitish line at its center (Fig. 5h). 4th: similar to 3rd but dorsal line diffuse white, bordered by thin green line, with dark green circular depressions visible on S6–S7 (Fig. 5i). 5th: length 12 mm; green circular depressions visible on S5–S8, hairs around posterior segments end in small globular structures. We observed only the 4th and 5th instars in the field and these were attended by *Crematogaster* ants. A DNO and TOs were present in all instars although it is not certain if they were functional in the first 2 instars.

Duration of immature stages based on 3 specimens: egg (4 d); 1st instar (2 d); 2nd (3 d); 3rd (5 d); 4th (4 d); 5th (7 d); 2 days to pupate; pupa (8–11 d).

Members of the genus *Spindasis* are reported to use a wide range of larval food plants (Igarashi & Fukuda, 2000; Veenakumari *et al.*, 1997) and to have an association with a particular species of ant. Egg laying is assumed to be driven by the need to have the correct ant association. Since this study shows that the presence of ants is not a mandatory condition for oviposition or for normal development of the larva and pupa of *S. vulcanus fusca* under lab conditions, *ex-situ* breeding for this species for conservation purposes could be carried out successfully if necessary.

However, our field observations clearly indicate that females fly around and settle only on plants that have ants. At one location, we observed many females flying in and out of a large patch of the larval food plant over a 2-hour period from about 11am but no oviposition took place. The temperature at this time was around 32°C and ants were not active on the surface of the plants, but were present at the base of the plants amidst the leaf litter and dead wood. On further examination, several larvae and pupae were found inside tightly closed shelters which the larvae constructed by folding a few leaves together with silk. These shelters also contained 5–10 *Crematogaster* ants (species not identified) that were attending the larvae and pupae. No immature stages of the ant were found inside the shelter. The ants moved in a frenzied manner over the plant surface after the shelters were opened up (Fig. 5j).

A female kept in captivity laid eggs freely after 3 days without any ants and all the eggs hatched without ants. Some of these larvae developed normally without ants up to the 3rd instar at which point a local ant (*Tapinoma melanocephalum*) (Fig. 5k) invaded the lab and gained access to the containers holding the larvae and immediately attended to them. *T. melanocephalum* is a very small (1.5 mm) widespread, tropical ant that can become a household pest. It is fond of sugar and occasionally eats dead or live insects. It is not clear how the *Tapinoma* ants found the *Spindasis* larvae though they forage widely. There is no evidence of lycaenid larvae using volatile chemicals for long-range attraction of ants, and the interaction of ant and larva relies on substrate-borne vibration signaling and contact chemoreception (i.e. the ant needs to physically touch the larva to pick up a signal (K. Fiedler, pers. comm.). One of the larvae collected in the field (possibly 4th or 5th instar) did not have ants in its shelter but looked healthy and normal. Nevertheless it died 2 days later. It is possible that the ants kept away from this larva because it was diseased



and was unable to produce the communication signals to maintain the association.

Both the field ants (*Crematogaster* sp.) and the lab ants (*T. melanocephalum*) were exceptionally excited around the larvae and ran about the shelter and container frantically, stopping frequently just behind the head at the junction of S2–S3 (Fig. 5l) and at the posterior end at the DNO. In the field and in the lab, the ants were periodically touched by the evversible tentacles of the TOs when they came to feed on the exudates of the DNO (Fig. 5m). It is not clear whether this behavior was to repulse the ants or to scent the ants for recognition. Ants attended the pupae as well.

The general habits of *S. vulcanus* in India described by de Nicéville (1890) apply to *S. vulcanus fusca* in Sri Lanka as well with some exceptions. The larva conceals itself by constructing a shelter which in the first 3 instars is not more than a partly folded up leaf. In the 4th and 5th instars the leaves or leaflets are brought together and held tightly with a small opening for the larva to exit to forage. The larva ventures out at night to feed but returns to its shelter during the day. At maturity, the larva pupates within the shelter constructed last. It does not seem to wander about in search of a place to pupate. The ants enter into the shelter built by the larva to attend to it. No ant nests were observed on the above-ground parts of the plant though the ants were observed moving up and down the stem of the plant to its base.

A parasitic ichneumonoid wasp (family Ichneumonidae, species not identified) emerged from a pupa collected from the field (Fig. 5n, o). Another hymenopteran larval parasitoid was recorded in *S. vulcanus* in India (Sidhu, 2010).

Larval food plants: In Sri Lanka, d'Abrera (1998) reported that it fed on *Ixora clerodendron* [sic] though there is no such species. This record likely comes from confusing two records from India: that of de Nicéville (1890): "The larva in Calcutta feeds on *Clerodendron siphonanthus*"; and of Swinhoe (1911–1912): "Grote bred this species in Calcutta on *Ixora longifolia*." Woodhouse (1949) also quoted larval food plant

Figure 5. Early stages of *Spindasis vulcanus fusca* from Sri Lanka. **a)** Larva, fifth instar, attended by ants in the field. **b)** Pupa, green form, dorsal view. **c)** Pupa, black form, lateral view. **d)** Egg, day before hatching. **e)** Larva, first instar, dorsal view. **f)** Larva, first instar, lateral view. **g)** Larva, second instar, dorsolateral view. **h)** Larva, third instar, dorsal view. **i)** Larva, fourth instar, dorsal view. **j)** *Crematogaster* ant. **k)** *Tapinoma melanocephalum* attending larva in the lab. **l)** *Crematogaster* ant attending to anterior end of larva. **m)** Ichneumonid pupal parasitoid, female. **n)** Ichneumonid pupal parasitoid, female, wing. **o)** Ichneumonid pupal parasitoid, female, wing.

information from Bell (1919) which is based on Indian sources. The current study showed for the first time that one of the larval food plants in Sri Lanka is *Cardiospermum halicacabum* (Sapindaceae), a plant completely unrelated to the genera *Ixora* (Rubiaceae) and *Clerodendrum* (Lamiaceae). The larvae feed on the leaves.

S. vulcanus fusca is widely distributed in the arid, dry and intermediate zones but scarce in the wet zone and hills. *Cardiospermum halicacabum* is common in the forests and waste lands in the moist and dry regions at lower elevations (Dassanayake, 1998). The distribution of this plant matches the distribution of the butterfly in that it has been found where the butterfly has been recorded. However, given the dependence of the butterfly on ants, its distribution is also likely constrained by the distribution of appropriate host ants. Since the members of the genus *Spindasis* tend to feed on a wide range of plants, it is likely that other larval food plants are used as well. For example, *S. v. vulcanus* has been reported in India as feeding on Rhamnaceae, Rubiaceae, Myrtaceae, Sapindaceae and Lamiaceae (Atluri *et al.*, 2012; Chowdhury *et al.*, 2009; Sidhu, 2010).

Spindasis schistacea (Moore, 1881). Plumbeous Silverline & *Spindasis nubilus* (Moore, [1887]). Clouded Silverline. Endemic.

The immature stages and larval food plants of these two species in Sri Lanka have not been described. In the course of this study, neither eggs, larvae nor pupae have been encountered. Adults of *S. schistacea* have been seen at a few widespread locations including Corbet's Gap, Kumbukgolla and Rambuk Oluwa (H. D. Jayasinghe, pers. comm.) and Elevankulam, Puttalam, Ritigala, Wasgamuwa and Riverston Knuckles (pers. obs.). Adults of *S. nubilus* have not been encountered though it has been historically reported from Giant's Tank, Jaffna and Elephant Pass.

Spindasis ictis ceylonica Felder, 1868. Ceylon Silverline. Endemic subspecies.

The immature stages and larval food plant of *S. ictis ceylonica* are described here for the first time.

Egg: pale green when freshly laid but turned dark brown within a day; disc-like, more or less flattened, not heavily sculptured like those of *S. vulcanus fusca*; micropylar end with a circular depression. 1st instar: Not recorded. 2nd, 3rd & 4th: similar to the 5th (Figs. 6a-d). 5th: Head black. Body salmon-colored, frosted in appearance, covered with minute white, black or dark brown funnel-shaped projections which give the larva its coloring and pattern; S3-S10 with subdorsal

white, inverted-cone-shaped protuberances; dorsal line from S6-S9 with concentric white and gray rings with brown centers; outer edges of dorsal line reddish-brown, sometimes obscure; lateral line broad, wavy, irregular and dark reddish-brown; spiracular line similar but less wavy; S2 dark brown dorsally and projects forward to cover much of the head; dorsum of S2 suffused with minute funnel-shaped black projections anteriorly and white towards the center and back; S3 black and densely packed with minute funnel-shaped projections; S11-S14 with a continuous black patch dorsally extending to the subdorsal line; S11 with a well-developed DNO; S12 with a pair of eversible TOs placed within the center of a cone-shaped projection that is rimmed with 5-6 clubbed filaments; between the TOs, four similar, shorter, clubbed filaments; anal plate light gray with minute dark-colored depressions and connected to the flange by 5 irregular lines that radiate out from the periphery of the gray center; numerous white filaments of even width extend out from the body along the flange and just below (Fig. 6d). Pupa: similar to that of *S. vulcanus fusca* (Fig. 6e). *S. ictis ceylonica* is multibrooded: larvae have been found at the same location in June, July and November. Duration of immature stages (based on observations of 3 specimens): 4th (11 d); pupa (9-11 d).

Larval food plants: One of the larval food plants in Sri Lanka is *Acacia eburnea* (Fabaceae-Mimosoideae); the larvae fed on the leaves. The larvae were found in the field in the company of *Crematogaster* ants (species not identified) at two locations: at Arippu (8°48'14.4"N, 79°56'22.6"E, 1 m asl) on the west coast in the Mannar district (Fig. 6f) and at Chundikulam (9°28'40"N, 80°35'38"E; 1 m asl) in the Jaffna district on the northeast coast (Fig. 6g).

The *Crematogaster* ants were small (3.5 mm long), black and reddish-brown. They formed colonies inside the paired thorns of *Acacia eburnea* (Fig. 6h), which are usually fused at the base. The thorns are filled with a corky material even when mature. It is likely the ants that remove the interior pith to create space inside the thorns as no hollow thorns were found without an ant entrance hole. Each colony comprises about 50-80 ants with eggs, larvae and pupae in the largest thorns of the Arippu population in which the largest thorns measured 80 mm in length and 11 mm in diameter at the base. At Chundikulam, the colony comprises about 20-25 in the largest thorns which measure 60 mm in length and only 7 mm in diameter at the base. The ants enter and exit from the thorns through a small hole (one hole per paired thorn, 1-2 mm in diameter) that is placed almost anywhere on the thorn but usually near the

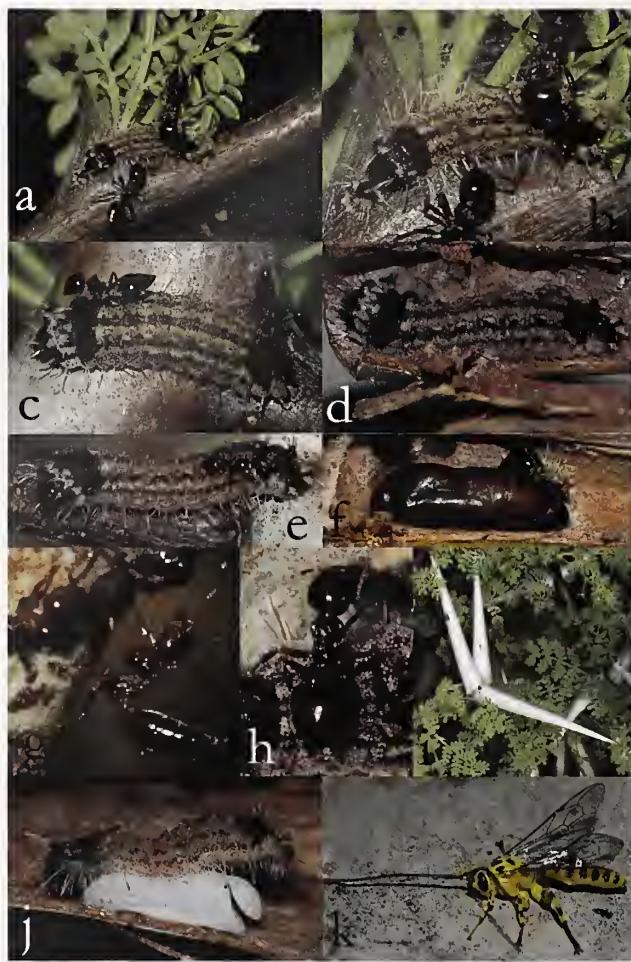


Figure 6. Early stages of *Spindasis ictis ceylonica* from Sri Lanka. **a)** Larva, second instar, dorsal view, with its larval nest and *Crematogaster* ants. **b)** Closeup of second instar larva. **c)** Larva, third instar, dorsal view. **d)** Larva, fourth instar, dorsal view. **e)** Larva, fifth instar, dorsolateral view. **f)** Pupa inside hollow thorn, lateral view. **g)** *Crematogaster* ant from Arippu. **h)** *Crematogaster* ant from Chundikulam. **i)** Paired thorns of *Acacia eburnea* with ant nest entrance hole visible in the center. **j)** Early instar larval skin with parasitoid pupa below from which the parasitoid has emerged. **k)** Ichneumonid parasitoid, male, emerged from pupa.

base. The ants were observed feeding on the glands on the petiole of *A. eburnea* and on dead insects at the base of the plant. They are not aggressive though they swarmed out when their colony was disturbed. Their sting is very mild.

S. ictis ceylonica females usually oviposited next to the hole of the ant nest in the thorn. On hatching, the larva consumed part of the eggshell. Usually one or two larvae, often of different instars, were found inside the thorns with ants attending them, and sometimes with one or two pupae as well. Larvae

were observed exiting the ant nest near sundown and during heavy cloud cover. Early instar larvae were observed making nests using silk to bind a few leaves together or with silk at the fork of small twigs, particularly when about to molt (Fig. 6a). The ants attended the *Spindasis* larvae assiduously, and fed on the fluid exuded by the DNO on the 11th segment. The ants were also active on the dorsum of S2–S3 from where they appeared to obtain some nutrition. The larva had the habit of quickly evert its TOs and touching the ants with the everted filaments when the ants were close to the DNO or TOs.

As the larva grew bigger, it made the entrance to the ant nest larger by chewing away the sides of the hole but the ants sometimes glued small pieces of chewed material to make the hole smaller again, only to have it enlarged again by the larva. The partition between the paired thorns eventually disappeared, perhaps chewed away by the larvae and/or ants, thus expanding the space available within. In the field, we found pupae inside hollow thorns with their heads pointing towards the entrance hole, usually within 1 cm of the hole. Usually one pupa was found per thorn but occasionally two, one on either side of the ant entrance hole. In the lab, the larvae pupated inside hollow thorns that were provided to them.

In the Chundikulam population, an early instar larva was found that had been parasitized though the parasitoid had already emerged (Fig. 6i) and a parasitoid wasp (family Ichneumonidae, species not identified) emerged from a pupa (Fig. 6j). Neither larval nor pupal parasitoids were found in the Arippu population.

S. ictis ceylonica is common in the plains of the dry and arid zones. It is also found in the drier hills of the eastern slopes up to about 1500 m asl in the Uva province in places such as Haputale and Bandarawela where the largest specimens and only the dry season form occur.

Acacia eburnea is a shrub or small tree found in very dry areas along the northwest coast from Anavilundawa to Jaffna and thence along the northeast coast to Yala in the southeast corner of the island (Dassanayake, 1980). The plant is absent in the drier hills of the Uva province and the inland areas of the dry zone. *S. ictis ceylonica* must therefore feed on other plants in these areas since the butterfly is not known to take part in flights and so must be breeding there. The distribution of the *Crematogaster* ant is unknown.

Spindasis elima fairliei Ormiston, 1924. Scarce Shot Silverline. Endemic subspecies.

The immature stages and larval food plant of *S. elima fairliei* are described here for the first time.

Egg: similar to that of *S. ictis ceylonica* (Figs. 7a, b). 1st instar: Head shiny black. Body light purplish-red with a faint white dorsal line; S2 large, rounded on the sides and slightly raised above other segments with a rounded, dark shiny dorsal patch; S3 and S4 dark on the dorsum; S4–S10 with whitish subdorsal diffuse spots that become smaller posteriorly; S12 with a short dark transverse band; S13 with a circular dark area on the dorsum; S2–S3 and S11–S14 with long black setae with white tips; S4–S10 with short, black setae arranged in transverse bands (Fig. 7c). 2nd: similar to the 1st except for the following: body slightly darker purplish-red covered with minute funnel-shaped projections; obscure white lateral line; large black patch on dorsum of S2 more prominent; DNO on S11 more clearly marked and fringed with 2–3 black filaments; S12 with a pair of TOs between which are two rows of black, slender, oblong filaments; setae absent; body covered with black club-shaped projections with brownish tips (Fig. 7d). 3rd: similar to the 2nd but in addition, minute, short-stalked, white, funnel-shaped projections on S2–S12; dorsal shield on S2 well defined, very dark and raised with a ridge along the dorsal line; S3 and S11 darker dorsally than the remaining segments except S2 (Fig. 7e). 4th: similar to the 3rd (Fig. 7f). 5th: similar to the 4th but with the following differences: body paler in colour—a light brownish-purple and more heavily speckled with funnel-shaped projections so that the larva appears frosted; translucent or white filaments along the edge of flange; spiracles light brown and indistinct (Fig. 7g). Pupa: similar to that of *S. vulcanus fusca*; 11 mm long, 3.5 mm wide (Fig. 7h). Duration of immature stages (based on observations of 4 specimens): 1st instar (4 d); 3rd (5–7 d); 4th (14 d); 5th (20 d); pupa (11 d).

Larval food plants: One of the larval food plants in Sri Lanka is *Acacia eburnea* (Fabaceae-Mimosoideae); the larvae fed on the leaves. The larvae were found in a field at Arippu, Mannar in the company of a species of *Crematogaster* ant that is yet to be identified. *S. ictis ceylonica* larvae were also found in the company of the same ant on the same bush and sometimes even within the same thorn.

See *S. ictis ceylonica* for a description of the ants, the behavior of the larvae and the distribution of *A. eburnea*. The only difference noted was that *S. elima fairliei* females were less discriminating and oviposited on any part of the plant as long as the ants were active nearby.

S. elima fairliei is found in the northern province and along the northwest coast of the arid zone. A few stray south along the northwest coast into the dry zone as far south as Anavilundawa. It inhabits the



Figure 7. Early stages of *Spindasis elima fairliei* from Sri Lanka. **a)** Egg, first day. **b)** Egg, second day. **c)** Larva, first instar, dorsal view. **d)** Larva, second instar, dorsal view. **e)** Larva, third instar, dorsal view. **f)** Larva, fourth instar, dorsal view. **g)** Larva, fifth instar, dorsal view. **h)** Pupa, dorsolateral view.

thorn scrub of the arid zone and the scrub jungle of the dry zone. It is not rare but is often mistaken for *S. ictis ceylonica*.

We have recorded the larvae of *S. elima fairliei* feeding only on *A. eburnea* and have found the plant wherever the butterfly has been recorded. However, given the propensity for larvae of the genus *Spindasis* to be polyphagous, there may be other larval food plants.

Spindasis lohita lazularia (Moore, 1881). Long-banded Silverline.

The final instar larva and pupa of *S. lohita lazularia* in Sri Lanka were described briefly by Moore (1880) and d'Abrera (1998) while Green (1902) briefly described its association with *Crematogaster* ants. Woodhouse (1949) quoted the description of the immature stages of *S. lohita* in India from Bell (1919). The immature stages of other subspecies of *S. lohita* in other countries have been fairly well documented (e.g. in Taiwan by Igarashi & Fukuda, 2000; in Singapore by H. Tam, 2010) and its larval food plants recorded (e.g. in the Andamans by Veenakumari *et al.*, 1997; in Malaysia by K. Fiedler, pers. comm.). *S. lohita* is a prime example of an obligate association with *Crematogaster* (in Malaysia: *C. dohrni artifex*), coupled with wide-ranging polyphagy (K. Fiedler, pers. comm.). In the course of this study, neither eggs, larvae nor pupae have been encountered but adults have been seen at several locations in all climatic zones including Atweltota, Elevankulam, Monaragala and Matale (H. D. Jayasinghe, pers. comm.) and Knuckles, Morningside and Puttalam (pers. obs.).

CONCLUSIONS

The immature stages of the genus *Spindasis* in Sri Lanka have been incompletely documented and many assumptions have been made particularly about the relationships of the larvae and pupae to the ants. These assumptions need to be revisited in light of the findings of the studies presented here. The life-cycles of all species deserve to be studied more carefully and in-depth. Similarly, with the exception of some species of *Spindasis* from Africa, the life cycles of other Asiatic *Spindasis* need to be better studied since only *S. lohita*, *S. syama* and *S. takanonis* are reasonably well covered.

ACKNOWLEDGEMENTS

Thanks to Krushnamegh Kunte for valuable information; Blanca Huertas, David Lees and Geoff Martin of NHM (London) for help with specimens and other information; NHM London for permission to use photographs of the type specimen of *S. greeni*; Channa Bambaradeniya and IUCN Sri Lanka and Devaka Weerakoon and the University of Colombo for administrative and logistical support; H. D. Jayasinghe, C. de Alwis and S. Sanjeeva for field support; the Department of Wildlife Conservation and the Department of Forestry, Sri Lanka for permission to do this research and field support; Nadeera Weerasinghe, Gehan de Silva Wijeyeratne and Jetwing Hotels for field support; R. K. Sriyani Dias of Kelaniya University for the identification of *Crematogaster rothneyi*. Konrad Fiedler has provided valuable information and a critical reading of the manuscript. All photography by the first author unless otherwise stated (Fig. 1a: Nadeera Weerasinghe; Fig. 2b: H. D. Jayasinghe).

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Este libro se terminó de imprimir
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